

Sequence Analysis and Antigenic Cross-reactivity of a Venom Allergen, Antigen 5, from Hornets, Wasps, and Yellow Jackets¹

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ABSTRACT. Ag 5 is a major allergen of vespid venom. The complete amino acid sequences of Ag 5 from two species each of hornets (*Dolichovespula arenaria* and *maculata*), wasps (*Polistes annularis* and *exclamans*), and yellow jackets (*Vespula maculifrons* and *vulgaris*) have been determined by amino acid sequencing and/or cDNA cloning. The sequence data reveal several conserved and variable regions for vespid Ag 5. The conserved regions show greater sequence similarity than do the less conserved regions to a family of proteins from human, mouse, and rat testis and to a class of pathogenesis-related proteins from tobacco and tomato leaves. Tests in the murine system for both T and B cell responses demonstrate a high degree of cross-reactivity of hornet Ag 5 with wasp or yellow jacket Ag 5 and a low degree of cross-reactivity of yellow jacket Ag 5 with wasp Ag 5. This pattern of cross-reactivity is in the same order as their sequence similarity. *Journal of Immunology*, 1993, 150: 2823.

Many stinging insects can cause allergy in humans. The most extensively studied is the honey bee, followed by the vespids, which include hornets, yellow jackets, and wasps (1, 2). Most vespid-allergic patients exhibit multiple reactions to more than one vespid venom by skin tests or antibody assays (3, 4). The multiple reactions are in part the result of partial cross-reactivity of venom proteins at the antibody level, as shown by studies in humans (4) and in animals (5, 6). The multiple reactions can also be a consequence of cross-reaction at the T cell level, so that susceptible persons respond to multiple vespids after exposure.

The vespids have similar venom compositions and their three major venom allergens are hyaluronidase (43 kDa),

phospholipase A₁ (37 kDa), and Ag 5 (23 kDa) (7–9). We previously cloned and sequenced two polymorphic forms of Ag 5 from white face hornet (*Dol m*³) and determined their disulfide bond pairings (10, 11). These two forms are designated as *Dol m* VA and VB according to a recently proposed nomenclature for allergens (12), and their sequences are given in Figure 1. The biologic function of Ag 5 in vespids is not known. *Dol m* V is found to have sequence similarity with a class of 15-kDa pathogenesis-related proteins from tomato and tobacco leaves (13, 14) and with a rat epididymal sperm-coating glycoprotein of 26 kDa (15).

We describe below the complete sequences of the homologous Ag 5 from yellow hornet (*Dol a*), yellow jackets (*Ves m* and *Ves v*), and wasps (*Pol a* and *Pol e*), designated *Dol a* V, *Ves m* V, *Ves v* V, *Pol a* V, and *Pol e* V, respectively. We also describe the cross-reactivity of these homologous proteins at T and B cell levels in the mouse system.

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³ Abbreviations used in this paper: *Dol m*, *Dolichovespula maculata*; *Dol a*, *Dolichovespula arenaria*; *Pol a*, *Polistes annularis*; *Pol e*, *Polistes exclamans*; *Ves m*, *Vespula maculifrons*; *Ves v*, *Vespula vulgaris*; PCR, polymerase chain reaction; RACE, rapid amplification of 3' cDNA ends.

<i>Ves m</i>	NNYCKIKCLKGGVHTACKYK SLKP	NCGNKkVVSyGLTKQEKQDILKE	1-47
<i>Ves v</i>	NNYCKIKCLKGGVHTACKYK SLKP	NCGNKvVVSyGLTKQEKQDILKE	1-47
<i>Dol a</i>	NNYCKI CpKG tHTLCKYGTSMKP	NCGgKIVKSYGVTNDEKNEIVKR	1-46
<i>Dol mA</i>	NNYCKIKCsrg IHTLCKFGTSMKP	NCGsKIVKvHGVsNDEKNEIVnR	1-47
<i>Dol mB</i>	NNYCKIKCrkG IHTLCKFGTSMKP	NCGrnVVKayGLTNDKNEIlkR	1-47
<i>Pol a</i>	VDYCKIKCPsG IHTVCQYGESTKPSKNCAGKVIKSVGPTTEEKKLIVSE		1-49
<i>Pol e</i>	VDYCKIKCPsG IHTVCQYGESTKPSKNCAGKVIKSVGPTTEEKKLIVSE		1-49

YCKI C G HT C G S KP NC G EK I

FIGURE 1. Amino acid sequences of vespid Ag 5. Ag 5 were isolated from yellow jackets (*Ves m* and *Ves v*), hornets (*Dol a* and *Dol m*), and wasps (*Pol a* and *Pol e*). The sequences given are in the order of *Ves m* V, *Ves v* V, *Dol a* V, *Dol m* VA and VB, *Pol a* V, and *Pol e* V. Residues that differ in two adjacent sequences are given in lower-case letters. Residues that are common to all sequences are given on separate lines beneath the sequences; residues underlined or dotted represent conserved or variable regions, respectively (see text).

<i>Ves m</i>	HNDFRQKIARGLETRGNPGPPAKNMKNLVsDELAyIAQVWANQCCY	48-96
<i>Ves v</i>	HNDFRQKIARGLETRGNPGPPAKNMKNLVNDELAYvIAQVWANQCCY	48-96
<i>Dol a</i>	HN ~~e~~ FRQKVAGLETRGNPGPPAKNMNlLVNDELAKIAQTWANQcNf	47-95
<i>Dol mA</i>	HNqFRQKVAGLETRGNPGPPAKNMNVLVNDELAKIAQTWANQCsF	48-96
<i>Dol mB</i>	HNdFRQnVAGLETRGkPGPPAKNMNVLVNDELAKIAQTWANQcdF	48-96
<i>Pol a</i>	HNRFRQKVAGLETRGNPGPPAASDMNDLVNDELAHIAQVWASQCQFL	50-99
<i>Pol e</i>	HNRFRQKVAGLETRGNPGPPAASDMNDLVNDELAHIAQVWASQCQFL	50-99

HN FRQ A GLETRG PGQP A M LVW DELA AQ WA QC

<i>Ves m</i>	GHDTCRDVAKYQVGQNVALTGSTAAVYnDPVKLVKMWEDEVKDYnPKKKF	97-146
<i>Ves v</i>	GHDTCRDVAKYQVGQNVALTGSTAAKYdDPVKLVKMWEDEVKDYnPKKKF	97-146
<i>Dol a</i>	GHDQCRNTAKYpVGQNVIASTTGNsYqTMSyLlKMWEDEVKDYnPhkd1	96-145
<i>Dol mA</i>	GHDQCRNTeKYQVGQNVIASTTGNsYaTMSKLIeMwEnEVKDFnPKKgt	97-146
<i>Dol mB</i>	nHddCRNTAKYQVGQNIaIsSTtatqfdRpsKLlKqWEdEvTeFNyKvGl	97-146
<i>Pol a</i>	VHDKCRNTAKYpVGQNIa YAGGSnLPDvVSLlKLWEnEVKDFnYnTGI	100-147
<i>Pol e</i>	VHDKCRNTAKYpVGQNIa YAGGSkLPDvVSLlKLWEnEVKDFnYnTGI	100-147

HD CR KY VGQN A L WE EV N

<i>Ves m</i>	SeNn FLKIGHYTMVWANTKEVCGSikYIQEnWHKHylVLCNYGpSGNFqNEELYQTK	147-204
<i>Ves v</i>	SgNd FLKtGHYTMVWANTKEVCGSikYIQEKWHKHylVLCNYGpSGNFmNEELYQTK	147-204
<i>Dol a</i>	mhNn FSKVGHYTMVWGKTKEIGCGSVKYIENKWHThylVLCNYGpAGNYMnQpYIERK	146-203
<i>Dol mA</i>	igdnfFSKVGHYTMVWGKTKEIGCGSVKYIENnWHThylVLCNYGpAGNYMdpQPIYERK	147-205
<i>Dol mB</i>	qnsN FrKVGHYTMVWGKTKEIGCGSikYIEdNWyThylVLCNYGpGnGdnfQPIYERK	147-204
<i>Pol a</i>	TKQN fAKIGHYTMVWGKTKEIGCGSLKYmENnMQNHylICNYGpAGNYLgQLPYTKK	148-205
<i>Pol e</i>	TKQN fAKIGHYTMVWGKTKEIGCGSLKYIENkMQNHylICNYGpAGNYLgQLPYTKK	148-205

... F K GHYTMVW TKE GCGS KY HYL CNYGP GN Y K

Materials and Methods

Isolation and amino acid sequences of vespid Ag 5

These proteins were isolated as described (7-9, 16). Their partial sequences were determined by Edman degradation of whole molecules and CNBr or proteolytic fragments of reduced and carboxymethylated Ag 5 (6). The proteases used were endoproteinases Asp-N (Boehringer Mannheim, Indianapolis, IN) and Glu-C (Pierce Chemical Co., Rockford, IL). The conditions used for cleavage and purification of peptides were taken from References 10, 17, and 18. Protein sequencing was performed on a Milligen-Bioscience model 6625 Prosequencer (Millipore Corp, Marlborough, MA).

cDNA of vespid Ag 5

Total RNA were isolated from venom acid glands of vespids as described before (10). First-strand cDNA was synthesized from total RNA (5 µg) with oligo(dT)₁₇ plus R₁/R₀ primers (Table I), in a 50-µl volume, by using the cDNA synthesis system from GIBCO-BRL (Bethesda, MD).

Two different procedures for PCR were used to obtain Ag 5-specific cDNA from venom-specific cDNA. One procedure amplifies the Ag 5-coding region, by using the first-strand venom cDNA as the template (0.5 µl of cDNA syn-

thesis mixture) and the specific sense and antisense primers of Table I, as outlined in Figure 2A. Another procedure is the RACE described by Frohman and co-workers (19, 20). Briefly, the procedure involves two successive rounds of PCR to amplify the Ag 5-specific cDNA with nested primers, as outlined in Figure 2B. In the first round, the template is the first-strand venom cDNA (0.5 µl of cDNA synthesis mixture), the sense primer encodes the amino-terminal region of Ag 5, and the antisense primer is R₀. In the second round, the template is the first PCR product (1/1000th of the first-round PCR mixture), the sense primer encodes the internal region of Ag 5, and the antisense primer is R₁. The presence of the desired cDNA was tested by Southern blot by probing with an oligonucleotide containing an internal sequence of Ag 5 that is different from that used for PCR. PCR were carried out with GenAmp PCR reagent kit with AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT). The oligonucleotide primers or probes of Table I were synthesized on the basis of partial amino acid sequence data with two exceptions; oligonucleotides 11 and 12 were synthesized on the basis of partial cDNA sequence data.

PCR products contain single 3'-overhanging A-nucleotides (21) and they were used directly for cloning into a PCR vector with compatible T-nucleotide overhangs (Invitrogen Corp., San Diego, CA). The INVαF' strain of

Table 1
Oligonucleotides for cloning of vespid Ag 5

No.	Oligonucleotide ^a	Comment
1	N ¹ N Y C K I ⁶ CCA TGG TT AAC AAY TAY TGY AAR AT	Residues 1 to 6 of <i>Dol m</i> , <i>Dol a</i> , or <i>Ves v V</i> ; PCR sense primer or first PCR sense primer in RACE
2	* K ²⁰⁴ R E Y V P ¹⁹⁹ CCG GAA TTC CTA TTT TCT TTC ATA AAC CGG	Reverse complement of residues 199 to 204 of <i>Dol a V</i> ; PCR antisense primer
3	K ⁷² A P P Q P G ⁶⁶ CTT CGC TGG AGG CTG TGG TCC	Reverse complement of residues 66 to 72 of <i>Dol m</i> , <i>Dol a</i> , or <i>Ves v V</i> ; hybridization probe
4	G ¹⁷⁰ V E K T N A ¹⁶⁴ CC IAC YTC YTT IGT RTT IGC	Reverse complement of residues 164 to 170 of <i>Ves v V</i> ; PCR antisense primer
5	H ¹⁴ T A C K Y G ²⁰ CAC ACI GCI TGY AAR TAY GG	Residues 14 to 20 of <i>Ves v V</i> ; second PCR nested sense primer in RACE
6	AAG GAT CCG TCG ACA TCG ATA ATA CGA CTC ACT ATA GGG AT ₁₇	dT ₁₇ -R _i /R _o adapter primer for first-strand cDNA synthesis
7	AAG GAT CCG TCG ACA TC	R _o antisense primer for first PCR in RACE
8	GAC ATC GAT AAT ACG AC	R _i antisense primer for second PCR in RACE
9	V ¹ D Y C K I K C P ⁹ GTI GAT TAT TGY AAR ATI AAR TGY CC	Residues 1 to 9 of <i>Pol a V</i> ; PCR sense primer
10	T ¹⁶⁶ K G W V M ¹⁶¹ CGT AGA TCT GT YTT ICC CCA IAC CAT	Reverse complement of residues 161 to 166 of <i>Pol a</i> or <i>Pol e V</i> ; PCR antisense primer
11	T ¹⁵ V C Q Y G ²⁰ CGT GGA TCC ACA GTC TGC CAA TAT GG	Residues 15 to 20 of <i>Pol a</i> or <i>Pol e V</i> ; sense first PCR primer in RACE
12	V ¹⁰² H D K C R ¹⁰⁷ CGT GGA TCC GTA CAC GAC AAA TGC AGG	Residues 102 to 107 of <i>Pol a</i> or <i>Pol e V</i> ; second PCR nested sense primer in RACE

^a R, A or G; Y, C or T; I, inosine.

* Denotes stop codon.

bacteria that was supplied with the TA cloning kit did not grow well after being transformed with Ag 5-containing plasmid; therefore, the XL-1 Blue strain of bacteria (Stratagene, La Jolla, CA) was used in its place. Plasmids containing Ag 5-specific cDNA were isolated from appropriate clones by using the QIAGEN plasmid kit (QIAGEN, Chatham, CA). The cDNA were sequenced by using the Sequenase kit (United States Biochemicals, Cleveland, OH), and two or more clones were sequenced for each sample. Also, the majority of PCR products were sequenced directly before TA cloning.

Murine T cell responses

Four groups of four female BALB/c mice at 8 to 10 weeks of age (The Jackson Laboratory, Bar Harbor, ME) were immunized with vespid Ag 5. One group was used for each Ag 5, i.e., *Dol a V*, *Dol m VA*, *Pol a V*, and *Ves v V*. Each mouse was immunized i.p. with 0.2 ml of 10 µg/ml vespid Ag 5 in 0.05 M sodium phosphate buffer, pH 6.0, plus 5 mg/ml alum, at weeks 0 and 2. Their spleen cells were collected 9 to 11 days after the second immunization, and after treatment with 0.17 M NH₄Cl they were used for proliferation assays at concentrations of about 4 × 10⁶ cells/ml, in culture medium containing 1% normal mouse serum

(Bioproducts for Science, Indianapolis, IN) in place of the usual 10% FCS (22).

To obtain a T cell line specific for *Dol m VB*, groups of four female BALB/c mice were each immunized i.p. with 0.2 ml of an emulsion of equal volumes of 50 µg/ml *Dol m VB* and CFA. The spleen cells were collected 11 days later, treated with 0.17 M NH₄Cl, and then suspended in standard culture medium (22) to give 2 × 10⁷ cells/ml in the presence of 4 µg/ml *Dol m VB*. After 5 days of culture, the cells were recovered by density centrifugation over Ficoll-Paque (Pharmacia, Piscataway, NJ). The recovered cells were resuspended in culture medium to give about 1 × 10⁶ cells/ml, followed by addition of irradiated syngeneic spleen cells at 4 × 10⁶ cells/ml. After 7 to 10 days of rest, the cells were subjected to two more rounds of 4 days of stimulation and 7 to 10 days of rest under the following conditions: 5 × 10⁵ T cells/ml, 5 × 10⁶ irradiated syngeneic spleen cells/ml, and 2 µg/ml *Dol m VB*. The recovered cells were >96% CD4⁺ and <0.5% CD8⁺, when analyzed by immunofluorescence staining with phycoerythrin-labeled anti-CD4 and FITC-labeled anti-CD8 (Becton Dickinson, Mountain View, CA). Proliferation assays of long term cultured T cell lines were made in standard culture medium under the following conditions:

A. PCR with Ag5-specific sense and anti-sense primers

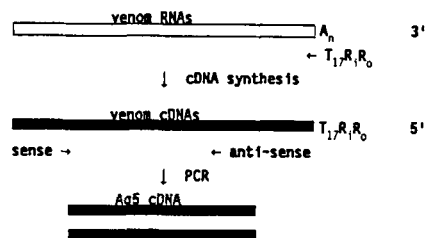
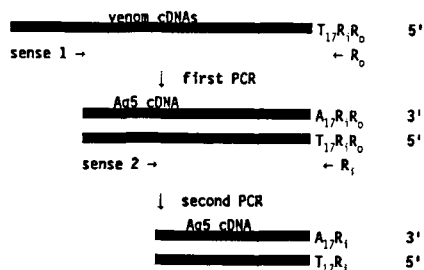
B. RACE with two Ag5-specific sense primers and R₀ and R_i anti-sense primers

FIGURE 2. Scheme for amplification of vespid Ag 5 cDNA. Venom cDNA were obtained from reverse transcription of venom RNA with oligo(dT)₁₇ plus R₀/R_i primers. The short arrows represent sense and antisense primers, whose structures are given in Table I.

2×10^5 T cells/ml, 2.5×10^6 irradiated spleen cells/ml, and 1×10^{-1} to 1×10^5 ng/ml Ag.

Murine B cell responses

Mice were immunized with vespid Ag 5 in the presence of alum, as described above for T cell responses, at weeks 0, 2, and 4. Sera were collected by retro-orbital plexus puncture and antibody titers were determined by solid-phase immunoassay (6).

Results

Amino acid sequence of vespid Ag 5

Multiple forms of hornet Ag 5 were isolated by ion exchange chromatography (7). Our published analysis of two forms of white face hornet Ag 5, *Dol m* VA and VB, showed them to differ by 23% in their sequences, as shown in Figure 1 (10). Similarly, two chromatographic forms were isolated for yellow hornet Ag 5. One form was found to have the amino-terminal sequence of *Dol a* V (Fig. 1) and the other form was found to have two additional amino-terminal residues, glutamic acid and threonine, which correspond exactly to those present in the leader sequence of *Dol m* VB. For wasp or yellow jacket Ag 5, only one chromatographic form was found for each protein.

The complete sequence of *Dol a* V was deduced from a cDNA sample that was amplified by PCR of venom cDNA with specific primers, as outlined in Figure 2. Our findings provide additional evidence that the two forms of *Dol a* V have the same primary sequence, with the only difference

Table II

Summary of peptides sequenced for *Ves v* and *Pol e* Vs

	Residues Sequenced	
	<i>Ves v</i> V	<i>Pol e</i> V
Intact protein	1-57	1-69
CNBr peptides		
A	74-97	1-10+
B	132-151+ ^a	76-112
C	160-204	161-177
Endo-Glu-C peptides		
A	61-81	43-79
B	83-102	85-130
C	104-118+	138-168
D		169-178
E		179-204
Endo-Asp-N peptides		
A	50-71+	126-139
B	103-124	
C	125-134	
D	135-138	
E	139-165+	

^a +, sequence continuing beyond the last residue.

being at their amino termini.

cDNA encoding two segments of *Pol a* or *Ves v* V were obtained from their respective venom cDNA by PCR with Ag 5-specific primers or by RACE with one Ag 5-specific primer and one R₀ or R_i antisense primer. The R₀ and R_i sites were introduced into the venom cDNA during the cDNA synthesis, as indicated in Figure 2. The complete cDNA for *Pol a* or *Ves v* V was assembled from their two segments and the translated protein sequences are given in Figure 1. The complete nucleotide sequences for *Dol a*, *Ves v*, and *Pol a* 5 have been deposited with GenBank.⁴

The sequences of another set of wasp and yellow jacket proteins, *Pol e* V and *Ves m* V, were obtained by Edman degradation of the intact protein and of its fragments that were generated by CNBr cleavage and by endoproteinase Glu-C or Asp-N digestion. A list of these overlapping peptides, which were used to establish the complete sequences, is given in Table II.

The sequences of *Ves m* and *Ves v* Ag 5 are almost identical, with only 10 of 204 residues differing. The sequences of *Pol e* and *Pol a* Ag 5 are also very similar, with only three of 205 residues differing. *Ves m* and *Ves v* are closely related species of the subgenus *Paravespula*, and *Pol e* and *Pol a* are members of the subgenus *Aphanilopterus*. *Dol m* and *Dol a* are of the same genus but their Ag 5 sequences have larger differences; *Dol a* V differs from *Dol m* VA and VB by 28 and 44 residues, respectively.

Comparison of the sequence data in Figure 1 shows that the cysteine residues are conserved in all vespid Ag 5. The disulfide bond pairings for *Dol m* VA and VB have been

⁴ The GenBank accession numbers for cDNA sequences of *Dol a*, *Ves v*, and *Pol a* Ag 5 are MM98859, MM98858, and MM98857, respectively.

FIGURE 3. Sequence similarity of vespid Ag 5, mammalian testis proteins, and plant pathogenesis-related proteins. Only portions of the sequences that have high degrees of sequence similarity are shown. The sequences are in the order of testis proteins from rat, human, and mouse, vespid Ag 5 from hornet (*Dol m* VA and VB) and yellow jacket (*Ves v* V), and plant pathogenesis-related proteins from tobacco (PR-1b) and tomato. Residues that differ in two adjacent sequences are given in *lower-case letters*. Residues that are identical for human testis protein and vespid Ag 5, or for tobacco protein and vespid Ag 5, are given on *two separate lines after the individual sequences*; *capital letters* indicate complete identity of all vespid proteins and *lower-case letters* indicate that only one of the proteins shares identity.

rat	PsgSdlLrvEwdhdavvNAQKWANrCiynHSplqhrTtLkCGENLfMan	57-106
human	PpaSnMLKMEWSrevTTNAQrWANKCtLqHSDpeDRKTstRCGENLYMSs	53-102
mouse	PtgSdlLKMEWSiqATTNAQKWANKCiLeHSSkdDRKiniRCGENLYMST	54-103
<i>Dol m</i> VA	PPAKNMNVLVWnDELAKIAQTWANQCsFgHDQCRN TeKYQVGQNVaIaS	69-117
<i>Dol m</i> VB	PPAKNMNVLVWnDELAKIAQTWANQCDfHHDdCRN TAKYQVGQNIaISs	69-117
<i>Ves v</i> V	PPAKNMkNLVWnDELAyvaQVWVWQcQyghDTCRD vAKYQVGQNVAltg	65-117
Tobacco	RAdVGVePLtWDngvAayAQNYvsQlAaDCNLvHShg QyGENLAqGs	14-61
Tomato	RAQVGvgPmsWDanLAAsrAQNYansrAgDCNLIHSga GENLakGg	15-59
	PPA NM W E AQ WAN C H G N ss	
	W A AQ Q Q G N A	
rat	yPaS WSSvIQdWYDEsLDFVfGfGPKkvG V kvGHYTQvVWnStf	107-150
human	DPTS WSSaIQSWYDEiLDFVYGVGPKsPNaV VGHYTQLVWYSTy	103-146
mouse	DPTL WStvIQSWYnEneDFVYGVGaK PNsa VGHYTQLVWYSTf	104-146
<i>Dol m</i> VA	TTgnsyatmsSKLIemWENEVkdFNpKKGtgdNnFSKVGHYTQMVWGKTK	118-167
<i>Dol m</i> VB	TTAtqfdrPSKLIKQWEDEVteFNyKvGLqnsN FrKVGHYTQMVWGKTK	118-166
<i>Ves v</i> V	sTAakyddPvKLVMWEDEVkdYNpKkKfsgnd FLKtGHYTQMVWanTK	118-166
Tobacco	GDFmT aaKAVeMVDEkqyYd hdsNtCaqGqvCGHYTQVWvNsv	62-106
Tomato	GDF T graAVqlWVsErpsYn yatNqCvgGkKCrHYTQVv	60-98
	s i w dE df vg n vGHYTQ VW t	
	k vemW dE y GHYTQ VW	
rat	lVaCGvAeCPdQ pLKfyfVChYCPgGNYvgRlys	151-184
human	qVGCgiAYCPNqDsLKyyYVCqYCPaGNNmnrKnt	147-181
mouse	kIGCGiAYCPNqDnLKYfyfVChYCPmGNNvmkKsT	146-181
<i>Dol m</i> VA	EIGCGSvKYIEEnWhThYLVCNYGPaGNYmdQPIY	168-202
<i>Dol m</i> VB	EIGCGSIKYIEENWYThYLVCNYGPGNdfnQPIY	167-201
<i>Ves v</i> V	EVGCGSIKYIQEKWhKHYLVCNYGPGNfNeeLY	167-201
Tobacco	RVGC aRvkCnNGgyvVSCNYDPPGNvIGqsPY	107-138
Tomato	RLGC gRaRCnNGwWfISCNYDPvGNwIGqrPY	99-130
	CGG k Y VC Y P GN mnQ	
	GC n CNY P GN Q Y	

determined (11) and it is most likely that all vespid Ag 5 have the same disulfide bond-pairing scheme.

Sequence searches in the Swiss Protein database, by using the FASTA program (23) at GenBank, revealed that vespid Ag 5 have sequence similarity to a protein from human or mouse testis (24), in addition to the previously noted rat testis protein and the plant proteins. In overlaps of about 130 amino acid residues, vespid Ag 5 have 28 to 35% residue identity with the mammalian testis proteins or with the plant proteins. These sequence similarities are shown in Figure 3 for *Dol m* VA and VB and *Ves v* V.

Inspection of the sequence data for vespid Ag 5 in Figure 1 reveals several conserved or variable regions that are hexapeptides or larger. By conserved region, we mean that the seven vespid Ag 5 in Figure 1 have the same residue or differ by a single substitution at each position. By variable region, we mean that the seven vespid Ag 5 differ by two or more substitutions at each position. The conserved regions appear to exhibit greater sequence similarity with the testis proteins from human, mouse, and rat and with the pathogenesis-related proteins from plants than do the other regions, as can be seen in Figure 3.

Murine T cell responses to vespid Ag 5

Responses were measured by proliferation assays with spleen cells from four groups of mice immunized with *Dol m* VA, *Ves v* V, *Dol a* V, or *Pol a* V. These results are given in Figures 4 and 5. In each figure are shown four separate proliferative responses of spleen cells upon stimulation

with the immunizing Ag 5 or the three homologous Ag 5. The multimodal and broad proliferative responses in these figures are not believed to result from experimental errors and probably represent populations of cells with different T cell epitope specificities. In each pattern of Figure 4 or 5, the concentrations of homologous vespid Ag 5 required for proliferative response are greater than or equal to that of the Ag that was used as the immunogen. The results in Figures 4 and 5A suggest that there is a greater degree of cross-reactivity of *Dol m* V, *Dol a* V, and *Ves v* V with each other than with *Pol a* V. However, the results in Figure 5B with *Pol a* V-specific spleen cells indicate an extensive cross-reactivity of *Dol m* V and *Pol a* V.

The results in Figures 4 and 5 were obtained by using *Dol m* VA as the immunogen as well as the stimulating Ag for proliferation. Similar results were obtained when a long term cultured T cell line specific for the other polymorphic form, *Dol m* VB, was used (Fig. 6).

Murine B cell responses to vespid Ag 5

Varying extents of cross-reactivity of vespid Ag 5 were found previously by solid-phase enzyme immunoassay with murine antisera (6). In Table III the efficiencies of autologous or homologous Ag 5 to stimulate secondary responses in mice primed with individual Ag 5 are compared. The results for groups A, B, and C suggest that *Ves v* V and *Pol a* V were nearly as effective as the autologous *Dol m* V in stimulating secondary antibody responses. The results for groups D, E, and F suggest that *Dol m* V was even more

FIGURE 4. Proliferation assay of spleen cells from mice immunized with *Dol m* VA (A) or *Ves v* V (B). The cells were stimulated with *Dol m* VA ($\times$), *Dol m* VB ($\circ$), *Dol a* V (∇), *Ves v* V (Δ), or *Pol a* ($\square$). The proliferative response in the absence of added Ag was about 2500 cpm.

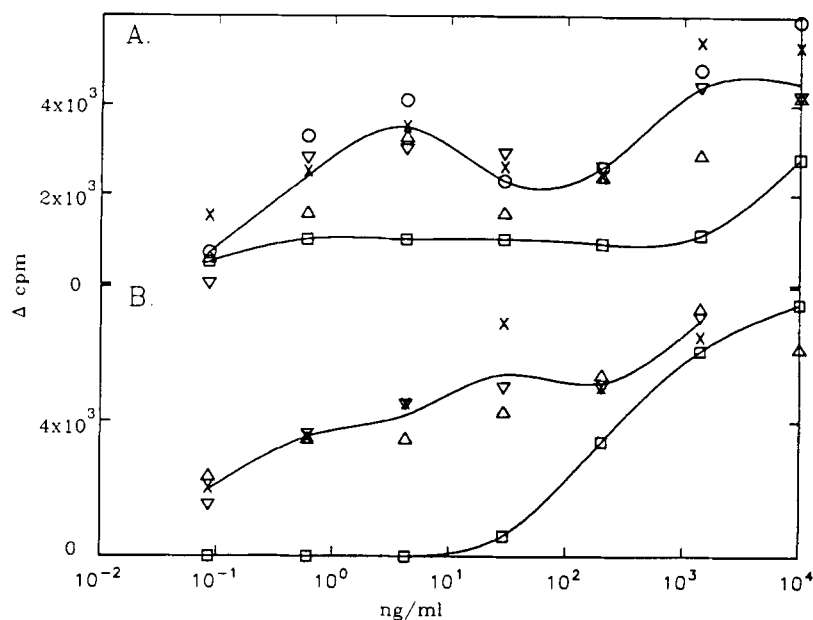
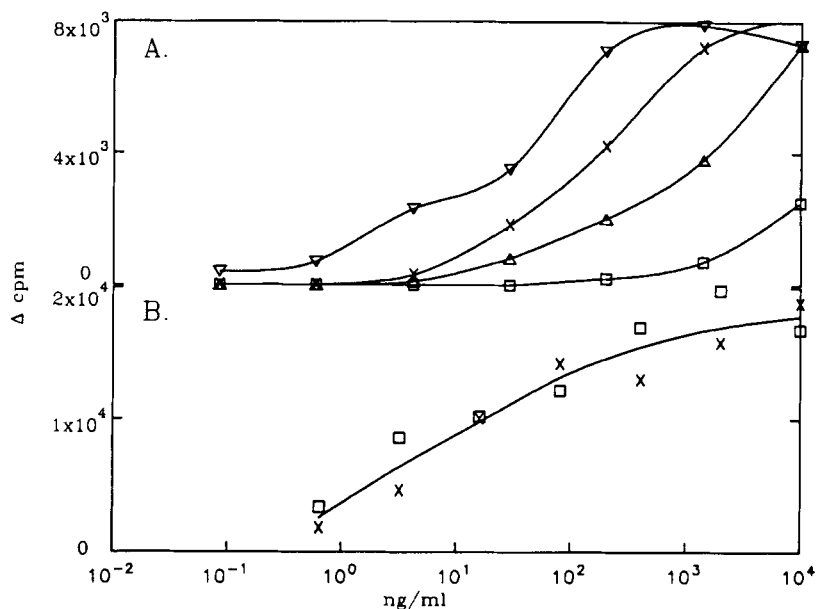


FIGURE 5. Proliferation assay of spleen cells from mice immunized with *Dol a* V (A) or *Pol a* V (B). The stimulating Ag used have the same symbols as in Figure 4. The proliferative response without added Ag was about 2500 cpm.



effective than the autologous *Ves v* V but *Pol a* V was ineffective in stimulating a *Ves v* V-specific secondary response. The results with groups G, H, and I suggest that *Pol a* V was effective for a *Dol m* V response but not for a *Ves v* V response. These results provide additional evidence that there is cross-reactivity of B cell epitopes of *Dol m* V with *Ves v* V or with *Pol a* V and relatively low cross-reactivity of *Ves v* V and *Pol a* V.

Discussion

Vespid Ag 5 show antigenic cross-reactivity at both T and B cell levels in the murine system (Figs. 4 to 6 and Table

III). There is greater cross-reactivity of hornet Ag 5 (*Dol m* VA and VB and *Dol a* V) with yellow jacket Ag 5 (*Ves v* V) or wasp Ag 5 (*Pol a* V) than of yellow jacket and wasp Ag 5 with each other. This pattern of cross-reactivity is in qualitative agreement with the degree of sequence similarity of these proteins (Fig. 1). When analyzed by the Genalign method (25), the alignment score decreases in the order of hornet-yellow jacket, hornet-wasp, and wasp-yellow jacket.

The major B cell epitopes of vespid Ag 5 are apparently of the topographic type, because their reduced and carboxymethylated derivatives showed very poor reactivity with murine antibodies specific for the native proteins (6).

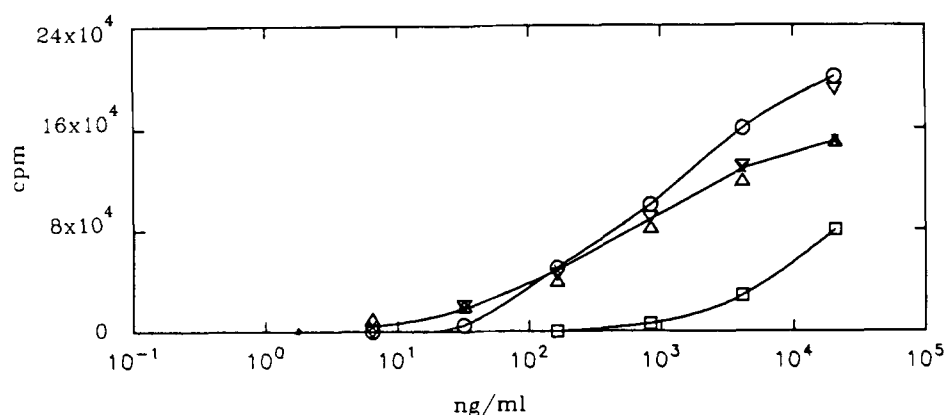


FIGURE 6. Proliferation assay of a long term cultured T cell line specific for *Dol m* VB. The stimulating Ag used have the same symbols as in Figure 4. The proliferative response without added Ag was about 120 cpm.

Table III
Immunogenicity of vespid Ag 5 for antibody responses

Group	Immunogen ^a		Antibody Titer for Primary Immunogen ^b		
	Primary	Boost	Week 3	Week 5	Week 5/ Week 3
A	<i>Dol m</i> VB	<i>Dol m</i> VB	3×10^3	9×10^4	30
B	<i>Dol m</i> VB	<i>Ves v</i> V	5×10^3	2×10^4	4
C	<i>Dol m</i> VB	<i>Pol a</i> V	9×10^2	9×10^3	10
D	<i>Ves v</i> V	<i>Dol m</i> VB	5×10^2	5×10^4	100
E	<i>Ves v</i> V	<i>Ves v</i> V	6×10^2	4×10^4	67
F	<i>Ves v</i> V	<i>Pol a</i> V	9×10^3	1×10^4	1
G	<i>Pol a</i> V	<i>Dol m</i> VB	5×10^2	8×10^3	16
H	<i>Pol a</i> V	<i>Ves v</i> V	3×10^2	6×10^2	2
I	<i>Pol a</i> V	<i>Pol a</i> V	8×10^2	4×10^4	50

^a Groups of four BALB/c mice were immunized with the primary immunogen at week 0 and week 2 and with the secondary immunogen at week 4.

^b Antibody titre represents reciprocal serum dilution for one-third maximal binding to solid-phase Ag.

However, their T cell epitopes are only dependent on the primary amino acid sequence, as is known for other protein Ag or allergens (26). This is confirmed by our unpublished results with recombinant hornet Ag 5 having free sulfhydryl groups in place of the disulfide bonds of native protein; the recombinant and the native proteins are very similar in their stimulation of T cell responses. Therefore, the present sequence data should be very useful for delineating the major common T cell epitopes of vespid Ag 5.

Antibody class switch events leading to synthesis of different isotypes are known to be under the control of Ag (or allergen)-specific T cells. Therefore, increased knowledge of T cell epitopes of allergens, such as vespid Ag 5, can contribute to our understanding of what makes an allergen. However, the T cell epitopes of known allergens do not appear to be unusual in their sequences, compared with those of other protein Ag (see Reference 26).

The sequence similarity of three groups of proteins from insects, mammals, and plants (Fig. 3) is of interest even though their regions of similarity are limited. The plant leaf proteins shown are members of the pathogenesis-related protein family that are formed on infection or physical in-

jury. Some have enzymatic functions and others have no known function, which is the case for the tobacco and the tomato proteins shown (13, 14). The rat sperm-coating glycoprotein is synthesized in the epididymis and is believed to assist in the maturation of sperm and in the penetration of the ovum by the fertilizing sperm (15). The testis-specific proteins from human and mouse have not yet been isolated and their biologic function is also unknown. They were identified by screening of a testis-specific cDNA library (24). Sequence comparisons of the rat, mouse, and human proteins suggest that the human and mouse testis-specific proteins are products of the same gene family but the rat sperm-coating glycoprotein is from a distinct but closely related gene family.

The sequence similarity of vespid Ag 5 to the mammalian proteins from testis may be related to the fact that the stinger apparatus of vespids is a modified ovipositor that no longer functions in egg laying (27). Possibly Ag 5 has an ancestral function in the promotion of ovum fertilization by sperm, as suggested for the rat protein. The sequence similarity of *Dol m* V to proteins from plant and mammalian sources is of interest, because a sizable proportion of people who have not been stung by insects are reported to have low levels of venom-specific antibodies (28, 29). In addition, we observed an association of *Dol m* VB- and tobacco protein PR-1b-specific IgG antibody levels in vespid-sensitive patients but not in normal blood donors (30). Further studies are required to establish whether these observations on sequence similarity are of significance in vespid allergy.

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