



IMMUNOCHEMICAL STUDIES OF STINGING INSECT VENOM ALLERGENS

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T. P. King. Immunochemical studies of stinging insect venom allergens. *Toxicon* **34**, 1455–1458, 1996.—Several major venom allergens from different insects of the Hymenoptera order have been cloned and sequenced by different laboratories. These insects include fire ants, honey bees, hornets, yellowjackets and wasps. These venom allergens have different biochemical functions, but have one feature in common, their varying extents of sequence identity with other proteins in our environment. Our studies in mice suggest that recombinant fragments containing regions of sequence identity of venom allergen(s) and host protein(s) show antigenic cross-reactivity. These studies lead to the hypothesis that cross-reactivity of venom allergens with host proteins promotes the immunogenicity of venom allergens in susceptible people. Copyright © 1996 Elsevier Science Ltd

Most common insect sting allergies are caused by insects of the Hymenoptera order, including honey bees, vespids and fire ants. The vespids include hornets, yellowjackets and paper wasps. It is estimated that 0.3–3% of the population has an insect allergy. It is also known that patients are frequently sensitive to multiple insects (Golden *et al.*, 1989; Reisman, 1994). The amount of insect venom injected in a single sting varies from $< 1 \mu\text{g}$ for fire ants to about $50 \mu\text{g}$ for bees. For vespids, this amount is about $10 \mu\text{g}$. Following exposure, susceptible people may develop sustained levels of venom-specific IgE and IgG antibodies. IgE antibodies are bound to mast cells and basophils. Cross-linking of cell-bound IgE antibodies by multivalent antigens or allergens results in the release of chemical mediators that trigger allergic responses.

Insect venoms are mixtures of proteins and peptides. Usually, the venom proteins are the active allergens. There is one exception, the honey bee venom peptide melittin, which is a weak allergen. Honey bee venom has three protein allergens: acid phosphatase, phospholipase A₂ and hyaluronidase (Hoffman, 1977; King *et al.*, 1976). Vespid venom has three protein allergens: phospholipase A₁, hyaluronidase and antigen 5 of unknown biological function (King *et al.*, 1978). Animal tests have shown that antigen 5 is not a toxin. Fire ant venom contains at least four known allergens, one of which has sequence identity with vespid antigen 5 (Hoffman, 1993). Almost all of the known venom allergens have been sequenced and/or cloned by different laboratories. Our own work has centered

mainly on the vespid allergens. We have cloned and expressed these proteins from yellowjacket, hornet and wasp venoms (Lu *et al.*, 1993, 1995; Soldatova *et al.*, 1993; King *et al.*, 1996).

A common finding from our sequence studies is the sequence identity of venom allergens with other proteins. Vespid antigen 5 has sequence identity with other proteins of diverse origins and tissues. These include proteins from fire ant venom (Hoffman, 1993), mammalian testis (Mizuki *et al.*, 1992), plant leaf (Cornelissen *et al.*, 1986), human brain tumor (Murphy *et al.*, 1994) and hook worm (Hotez *et al.*, 1995). The degree of sequence identity varies from 50% for the entire molecules of vespid and fire ant antigen 5 to 23% for portions of the vespid and the human brain tumor proteins. The plant leaf pathogenesis-related proteins and the brain tumor protein are both inducible on viral infections. Vespid phospholipases have no sequence identity with any known phospholipase, but one-third of the molecule has 40% identity with mammalian lipases. Vespid hyaluronidases have 50% sequence identity with the bee enzyme and 30% identity with a mammalian sperm protein.

It has been shown with some patient sera that the IgE antibody binding to solid-phase yellowjacket or bee venom can be inhibited equally by both venoms (Hoffman and Word, 1984; Reisman *et al.*, 1984). That is, the multiple sensitivity can be due to antigenic cross-reactivity of individual venom allergens. The sequence identity of vespid and bee hyaluronidases provides a structural basis for the observed antigenic cross-reactivity.

We next studied whether the sequence identity of venom allergens with host proteins may result in antigenic cross-reactivity. Normally, a host does not mount immune responses to self-proteins. It is possible that insect-allergic people recognize self-proteins so that they are primed to respond to the cross-reacting venom allergens. Figure 1 shows the sequences of fragments of hornet phospholipase (PLA) and mouse hepatic lipase (MHL) (Oka *et al.*, 1991), each fragment containing about 120 residues. The four underlined regions range in size from 9 to 15 residues; each region has 7 to 12 identical residues, which are shaded. These regions are long enough to be able to function as T-cell or B-cell epitopes.

The fragments shown in Fig. 1 were prepared by recombinant technology, and tested to ascertain whether the mouse lipase fragment could prime mice for antibody responses to hornet PLA fragment or the natural PLA (unpublished results). Pre-immunization of mice with mouse lipase fragment followed by PLA fragment immunization gave an approximately 50-fold higher PLA fragment-specific antibody titer than the control group did. Such a priming effect is not seen with mice immunized with the natural PLA molecule.

MHL 107	QHYTIAVQNTFRIVGQDVAALLLWLEESAKFSRSKVHLIGYSLGAHVSGFAG	157
PLA 99	--YKAAVGNTRLVGNFIAMI AKKLVEQYKVPMTNIRLVGHSLGAHISGFAG	147
MHL 158	--SSMDGKNKIGRITGLDPAGPMFEGTSPNERLSPDDANFVDAIHFTTREH	206
PLA 148	KRVQELKLGKFSEIIGLDPAGPSEKKNDCSERICETDAHYVQILHT-----	193
MHL 207	MGLS-VGIKQPIAHYDFYPNGGSFQPGCHFLE	237
PLA 194	--SNLGTERTLTGTVDIFYINNGSNQPGCRYI-	222

Fig. 1. Sequence identity of hornet phospholipase (PLA) and mouse hepatic lipase (MHL) fragments.

That is, the primed group did not show an increased antibody titer for the natural PLA molecule. The data suggest that the priming effect is not due to cross-reacting T-cell epitope(s). If this were the case, antibody responses to both the natural PLA and PLA fragments would have increased. The data suggest that the priming effect is due to any cross-reacting B-cell epitopes of the continuous type that are present in the natural PLA and its fragment. The fragment lacks the discontinuous epitopes of the natural PLA since it contains only part of the PLA molecule and it lacks the cross-linking disulfide bonds of PLA.

The above results provide partial support for the hypothesis that cross-reactivity of venom allergens with host proteins may promote the immunogenicity of venom allergens. This is of interest as we do not know why susceptible people are so readily sensitized by microgram amounts of venom allergens. Normally, when proteins are injected into experimental animals, strong immune responses are not obtained. Strong responses are reproducibly obtained only when proteins are given together with adjuvants.

Another area of interest is the use of T-cell epitope peptides for immunotherapy. These studies were made initially with the bee venom allergen melittin because of its small size, 26 amino acid residues. Tests with various peptide fragments established that melittin has only one B-cell and one T-cell epitope, located in the regions of residue 20–26 and 10–19, respectively (Fehlner *et al.*, 1991).

We found that subcutaneous pretreatment of mice with melittin T-cell epitope peptide led to partial suppression of melittin-specific antibody responses (unpublished results). Mice were pretreated s.c. with 20 or 60 nmoles of peptide on day –9, –8 and –7. They were then immunized i.p. with 2 nmoles of melittin and alum as adjuvant. Treated mice showed an approx. 80% decrease in melittin-specific antibody level compared to control. Spleen cells of the treated animals showed decreased proliferative responses on stimulation with melittin, about two-thirds of the control, which is suggestive of T-cell inactivation.

An attractive advantage of peptide immunotherapy over the current practice of venom therapy is its relative safety, as peptides are less likely than proteins to cause anaphylactic shock. However, there are potential problems (Geffer, 1992; Yssel *et al.*, 1994). Most allergens have multiple T-cell epitopes. Can antibody synthesis of a multivalent allergen be modulated by treatment with a single epitope? Two published reports suggest that this is possible, one on a cat allergen (Briner *et al.*, 1993) and the other a mite allergen (O'Hehir *et al.*, 1993). Another possible problem is major histocompatibility complex (MHC) restriction. MHC is a highly polymorphic molecule, and MHC molecules from individuals may bind different T-cell epitopes.

One possible way to circumvent these problems is to use a mixture of overlapping peptide fragments. Towards this objective, we have prepared a series of recombinant fragments encompassing the hornet antigen 5 molecule. These fragments range in size from 54 to 114 residues, and they are found to retain the T-cell epitopes of antigen 5 (King *et al.*, 1995). Preliminary tests (unpublished results) showed that suppression of antibody responses was observed in mice pretreated with individual fragments, but the extent of suppression is low—only about 20% compared with 80% suppression obtained with melittin. We hope to carry out further experiments to test whether a greater degree of antibody suppression can be obtained using a mixture of these fragments.

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