

IgE antibodies to bee venom, phospholipase A, melittin and wasp venom

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Summary

Specific IgE antibodies against bee venom, phospholipase A, melittin and wasp venom have been examined in fifty patients with an unusually severe reaction after bee or wasp sting.

Two thirds of the bee venom-sensitive patients also have detectable IgE antibodies to wasp venom. More than 50% of the wasp venom-sensitive patients are also allergic to bee venom. Phospholipase A and melittin IgE antibodies were found, respectively, in two thirds and one third of the bee venom-sensitive cases.

Specific IgE antibody determinations by the Radioallergosorbent test play an essential role in the diagnostic work. After a reaction to hymenoptera stings both bee and wasp venom tests are necessary due to the high incidence of a false or incomplete identification of the stinging insect.

Melittin, known for its potent pharmacological activity and possibly responsible for most of the side effects in bee venom immunotherapy, can probably not be excluded from therapeutic venom preparations since IgE antibodies to the melittin preparation were detected in one third of the cases.

Introduction

Bee- and wasp-venom allergic patients represent an increasing diagnostic and therapeutic problem.

The diagnostic problem is based on the fact that the hymenoptera is quite often wrongly identified, leading to a false case history. We therefore tested sera for IgE antibodies against bee venom (BV) and wasp venom (WV) and compared the result with the case history given.

The therapeutic problem is caused by the ineffectiveness of whole body extracts (WBE) (Hunt *et al.*, 1978; Light *et al.*, 1977; Urbanek & Karitzky, 1978). The side effects of bee venom (BV)-therapy, however, seem to occur more often than in therapy with WBE (Urbanek & Karitzky, 1978).

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Bee venom consists of a number of components, the peptides melittin and apamin, the proteins hyaluronidase and phospholipase A₂ (Hoffman & Shipman, 1976; King *et al.*, 1976). Furthermore two new components, the allergens B and C have been identified by gel electrophoresis (Hoffman, Shipman & Babin, 1977; Hoffman, 1977).

We have included two interesting allergens in our study: phospholipase A₂ and melittin, the former since it seems to be a major allergen in bee venom and is ideal for experimental work; the latter since it constitutes more than the half of the bee venom on a dry weight basis.

As with phospholipase A₂, melittin has a marked pharmacological activity (Vick & Brooks, 1972) and immunotherapy with a melittin-free venom preparation might reduce side effects in BV immunotherapy (Hunt *et al.*, 1978; Urbanek & Karitzky, 1978). We therefore examined sera from allergic patients for specific IgE antibodies to phospholipase A₂ and melittin in addition to the above mentioned venoms in order to study the frequency of melittin sensitized patients.

Material and methods

The serum samples of fifty patients with a positive history of adverse reactions to a bee and/or wasp sting were examined.

The serum was either analysed immediately after bleeding or stored deep frozen until analysis. IgE antibodies against bee (Honey bee, *Apis mellifera* L.) venom and wasp (Common wasp, Yellow jacket, *Vespula* spp.) venom were measured by Phadebas RAST®. The tests were run and results were evaluated according to the instructions for the use of the test.

Phospholipase A and melittin was provided by Sigma Chemical Corporation, St. Louis, U.S.A. The lyophilized material was checked for purity by gel electrophoresis. The patterns in the gel electrophoresis were compared with those gained from whole bee venom, which again was supplied by Sigma Chemical Corporation.

The sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis was performed essentially as described by Swank & Munkres (1971). The lyophilized antigens were dissolved in a concentration of 1 mg/ml in 0.01 M phosphoric acid, adjusted to pH 6.8 with tris(hydroxymethyl)aminomethane containing 1% SDS, 8 M urea and 1% 2-mercaptoethanol. The samples were stored at room temperature for 5 min and then heated at 100°C for 2 min.

The acrylamide gels contained 0.1% SDS, 0.075% N,N,N',N'-tetramethylethylenediamine (TEMED), 8 M urea and 0.1 M phosphoric acid at pH 6.8. The acrylamide concentration was 15% and the concentration of N,N'-methyleneethylenbisacrylamide was 0.5%.

When all components were dissolved, 0.07% ammoniumperoxodisulfate was added and gas was removed by evacuation of the vessel, after cooling with ice for 5 min. The gel was polymerized in glass tubes with isobutanol layered on top of the gel mixture. The electrode buffer was 0.1 M phosphoric acid at pH 6.8 containing 0.1% SDS. As a standard, 2 µl of bromphenol blue dye was added.

The allergen solutions were applied to the gel with a Therumo-syringe. The volume added was 50 µl and electrophoresis was run with 5 mA for 6 hr. The gels were stained for 1 hr in a bath which contained 15% methanol, 10% acetic acid and 0.05% Coomassie brilliant blue R-250. The dye was then removed by 15% methanol and 10% acetic acid.

The allergens phospholipase A and melittin were coupled to paper discs from

Pharmacia Diagnostics AB, Uppsala (Phadebas RAST Special Allergen Service) and IgE antibodies against phospholipase A and melittin were examined in the serum samples following the same procedure as described for Phadebas RAST. Total IgE levels were measured with Phadebas IgE PRIST®.

Results

Bee venom can be separated into several fractions with different physico-chemical and biological properties. Gel electrophoresis showed a multiplicity of proteins and peptides in the whole venom as well as in the purified preparations of phospholipase A and melittin (Fig. 1).

Out of the serum samples taken from fifty patients examined for IgE antibodies to bee and wasp venom, twenty-three were sensitive to bee venom, twelve were sensitive to wasp venom, nine did not have specific IgE antibodies in spite of a positive history of an unusually severe reaction after the sting, and six had a history of a bee sting, but IgE antibodies to wasp venom were found. Total serum IgE was normal in about 80% of the individuals, as shown in Table 1. The range of symptoms which occurred after the sting are listed in Table 2. Combinations of symptoms did occur. The comparison between RAST classes and clinical symptoms is given in Table 3. The correlation is good, but exceptions did occur.

The twenty-three bee venom-sensitive patients (as related to case history) did have IgE antibodies to phospholipase A in sixteen cases and to melittin in seven cases. An

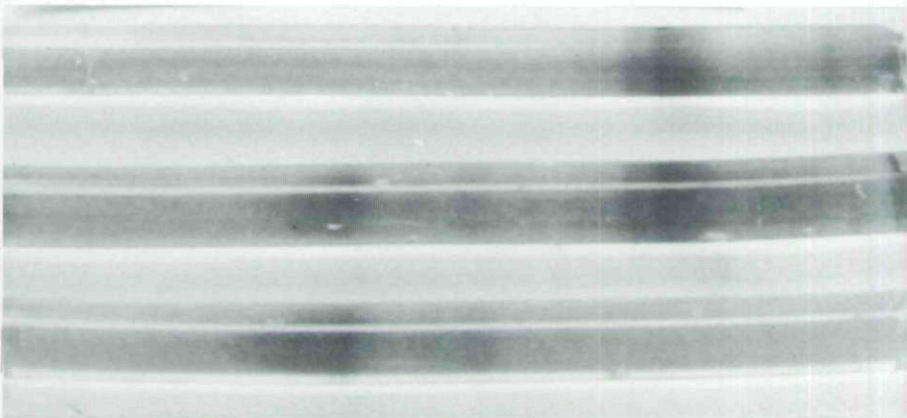


Fig. 1. Gel electrophoresis with phospholipase A (top), bee venom (center) and melittin (bottom).

Table 1. Total IgE-levels in bee and/or wasp venom-allergic patients. Determination by PRIST ($n=50$)

IgE level (u/ml)	No. of patients	%
0-20	11	22
21-100	20	40
> 100	19	38

Table 2. *In vivo* symptoms ($n=50$)

Symptom	No. of patients
Marked local swelling	22
Generalized urticaria	15
Bronchospasm	15
Syncope	9

Table 3. Comparison between RAST classes and clinical symptoms in bee venom-sensitive people ($n=23$)

Symptoms	No. of reactions				
	RAST classes				
	0	1	2	3	4
Marked local swelling	0	0	7	4	1
Generalized urticaria	1	0	6	5	
Bronchospasm	0	0	6	6	
Syncope	1	0	2	3	

Table 4. RAST positive (class 1 or above) results in bee venom-sensitive patients ($n=23$)

	No. of positive results	RAST class			
		1	2	3	4
Bee venom	23	0	12	10	1
Phospholipase A	16	4	5	6	1
Melittin	7	3	3	1	0
Wasp venom	17	3	8	4	2

Table 5. RAST positive results in wasp venom-sensitive patients ($n=12$)

	No. of positive results
Wasp venom	12
Bee venom	7
Phospholipase A	5
Melittin	0

Table 6. Patients with a positive history but negative RAST result ($n=9$)

Symptom	No. of negative results
Marked local swelling	7
Shiver	1
Urticaria	1

interesting finding is the high number (seventeen) of sensitivities to wasp venom (Table 4).

In twelve wasp-sensitive patients (as related to case history) IgE antibodies to bee venom were found in seven cases and to phospholipase A in five cases. However, no IgE antibodies to melittin could be detected (Table 5).

In the cases with positive history and negative RAST the corresponding clinical symptoms are given in Table 6.

Discussion

There are few people who have not been stung by a bee or wasp at some time in their life. Only a small number develop allergic reactions. It is still not understood why most people do not respond with IgE antibody production, while some do. In contrast to other allergic diseases like bronchial asthma and allergic rhinitis no genetic factors seem to be involved in bee or wasp venom-allergy. This means that even non-predisposed persons can develop an allergy to bee or wasp venom. This fact and the rather high number of fatalities after a sting in hypersensitive patients makes this type of allergy of general interest.

Moreover, since no additional 'shock organ' is necessarily affected, bee venom-allergy is of great interest as a research model for type I allergy. Clinical practice and *in vivo* and *in vitro* tests sometimes are controversial. Our findings reveal a high number of concomitant allergies to both venoms, although the patient's history is related to one allergen only.

Since no cross-reactivities between bee and wasp venom could be found (Arbesman, Langlois & Shulman, 1965; Kern *et al.*, 1976; Nair *et al.*, 1976; Rhoades, 1978) it is obvious that in these people additional allergy was detected by chance and, fortunately, they have not so far experienced *in vivo* challenge tests.

On the other hand one can expect that specific IgG antibodies present together with IgE antibodies might, under certain conditions, block the allergic reaction (Light *et al.*, 1977; Müller, Johansson & Streit, 1978; Urbanek & Kavitzky, 1978). This means that the presence of specific IgE antibodies at least with a low titre does not necessarily indicate clinically important allergy (Yunginger *et al.*, 1978).

These interpretations are possible in cases with specific IgE antibodies to both venoms.

In those patients where IgE antibodies to only one venom could be found and the history was positive to the other venom the insect obviously was wrongly identified while stinging. Consequently, in any case where history indicates sensitivity to an insect sting, IgE antibody determinations should be done to bee venom as well as to wasp venom.

Our findings with bee venom components show that IgE antibodies develop to a higher or lower degree to the different allergens.

IgE antibodies against phospholipase A could be found in two thirds (Arbesman *et al.*, 1976; Light *et al.*, 1976) and against melittin in one third of the cases (Paull, Yunginger & Gleich, 1977).

Although melittin possesses a potent pharmacological activity, and despite the fact that one out of three bee venom-allergic patients are sensitive, it is not possible to give melittin-free venom immunotherapy.

In conclusion, diagnosis of venom allergy should include determinations of specific IgE to both bee and wasp venom. Despite the variable sensitivities to components of bee venoms it seems reasonable to give whole bee venom for immunotherapy.

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