

Use of RAST Technique in Wasp Sting Hypersensitivity

Cross-Reactions between Various Insect Antigens are Specially Considered

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Clinical hypersensitivity to wasp stings was found to be fairly well correlated with the presence of serum IgE-antibodies against yellow jacket venom as detected by the RAST technique. Such antibodies were never found in a control group of non-allergic blood donors, but they were detected in a surprisingly large proportion of patients with bee sting allergy without known allergic reactions to wasps. Studies using RAST inhibition technique failed to prove cross-reactions between bee and wasp venoms.

Considerably better results were obtained when venom antigens instead of whole body antigens were used in the RAST. RAST inhibition studies suggested that IgE-antibodies detected with RAST using whole body antigen are directed against bee venom constituents in the whole body extract.

Key words: bee, wasp, hornet, venom; cross reactions; Hymenoptera hypersensitivity; RAST wholebody extracts.

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The value of the RAST technique (Radio-allergosorbent test) (11) in the diagnosis of bee sting hypersensitivity has been confirmed by several authors (7, 9, 12). Serum IgE-antibodies to bee venom are detected in up to 90% of patients with immediate hypersensitivity reactions to bee sting and only rarely found in non-allergic blood donors.

In our experience, case histories of patients with *Hymenoptera* hypersensitivity frequently suggest cross-reactions between insect antigens of different species. For example when a patient with known allergy to bee stings experiences a general allergic reaction following his first wasp sting. Misidentification of the stinging insect may, however, be the reason for these observations (6).

The objective of this study was to investigate the value of RAST in patients with

suspected immediate hypersensitivity to wasp stings and to look for *in vitro* cross-reactions between various insect antigens.

MATERIAL AND METHOD

Serum samples. The following populations were studied:

- 24 patients with probable or convincing and
- 14 patients with questionable anamnestic evidence for wasp sting hypersensitivity.

In 10 of these 34 patients there was anamnestic suspicion of hypersensitivity to wasp stings only, while in the remaining 28 both bee and wasp sting hypersensitivity was suspected. Also two patients reported adverse reactions following hornet stings.

- 22 patients with probable or convincing evidence of bee sting hypersensitivity without known allergic reactions to wasp stings and a positive RAST with bee venom.
- 10 atopic controls with pollinosis and no history of generalized allergic reactions to *Hymenoptera* stings. One individual had severe local reactions to insect stings.
- 10 non-atopic controls with no history of insect hypersensitivity.

Case history. A careful case history as regards reactions to *Hymenoptera* stings was collected for each participant and was classified as: (1) negative, no history of untoward reactions to insect stings; (2) questionable, adverse reaction not typical for an allergic reaction – possibly reaction of vasovagal nature or insect not verified; (3) probable, only one sting with subsequent allergic reaction; (4) convincing, more than one sting with subsequent allergic reaction.

The reaction time between sting and first allergic manifestations was less than 30 min in all patients. The time interval between blood sampling and the last sting lay within 6 months in 12 patients with probable or convincing wasp hypersensitivity, in six patients with questionable wasp hypersensitivity and in 18 patients with bee hypersensitivity.

Antigens. Honey bee venom (*Apis mellifera*=HB) was obtained from Sigma Chemicals, U.S.A. and Mack Nachf., Illertissen, BRD; venoms of yellow jacket wasp (*Vespa maculifrons*=YJ), white-faced hornet (*Vespa maculata*=WFH) and paper wasp (*Polistes fuscatus*=PW) from Dr. A. W. Benton, Pennsylvania, U.S.A.

12% whole body extracts (12 g of whole insect body extracted in 100 ml of Evans solution) of bee (BWBE) and the European wasp (*Vespa vulgaris*=WWBE) were kindly supplied by Mr. G. Green, Bencard Allergy Unit, England.

RAST. Phadebas RAST® bee venom discs (Pharmacia Diagnostics AB, Uppsala, Sweden) were used throughout this study. The additional venom discs were ex-

perimental discs meeting the same quality standards as the Phadebas discs. The whole body allergen discs were prepared by the same procedure from the stock extracts without further dilution. The other reagents used were those of Phadebas RAST and the technical procedure was the same as in the commercially available test. All test results were evaluated according to the Phadebas RAST reference system.

RAST inhibition studies using the RAST technique were performed according to the method described by Arbesman *et al.* (1). 50 µl of patient sera were incubated in duplicate with 150 µl of serial tenfold dilutions of insect antigens in 2% human serum albumin overnight at 4°C. The next day the appropriate disc was added and the RAST procedure continued as described for the direct RAST technique.

RESULTS

Fig. 1 and Table 1 show the results obtained with the various insect antigens in patients and controls.

With YJ 50% of patients with probable or convincing, 14% of those with questionable wasp hypersensitivity and 41% of those with bee sting hypersensitivity were RAST positive. Atopic and non-atopic controls were always negative. Due to the low frequency of stings by hornets and paper wasps only a few patients had antibodies to WFH and PW. 43% of the patients with probable or convincing and 36% of those with questionable wasp hypersensitivity had IgE-antibodies to bee venom. The two HB RAST positive atopic controls had both been stung by bees previously and one had suffered severe local reactions.

IgE-antibodies to BWBE were found in 41% of bee sting-allergic patients and to WWBE in 13% of patients with probable or convincing wasp sting hypersensitivity. In 12 patients of this latter group the blood sample was taken within 6 months after their last allergic reaction (Table 1). Seven of them had IgE-antibodies to YJ and an additional to HB.

Table 1

IgE-antibodies to *Hymenoptera* venoms in various populations

Population	Number	Positive RAST with				
		YJ	WFH	PW	HB	At least 1 antigen
Convincing wasp hypersensitivity (WH)	12	6 (50%)	1 (8%)	1 (8%)	7 (58%)	8 (67%)
Probable WH	12	6 (50%)	1 (8%)	1 (8%)	4 (33%)	6 (50%)
Convincing and probable WH studied within 6 months after the last sting	12	7 (58%)	1 (8%)	1 (8%)	8 (67%)	9 (75%)
Pure WH (convincing and probable)	10	5 (50%)	0	0	3 (30%)	5 (50%)
Questionable WH	14	2 (14%)	1 (7%)	1 (7%)	5 (36%)	5 (36%)
Probable or convincing bee hypersensitivity	22	9 (41%)	7 (32%)	4 (19%)	22 (100%)	22 (100%)
Atopic controls	10	0	0	0	2 (20%)	2 (20%)
Non-atopic controls	10	0	0	0	0	0

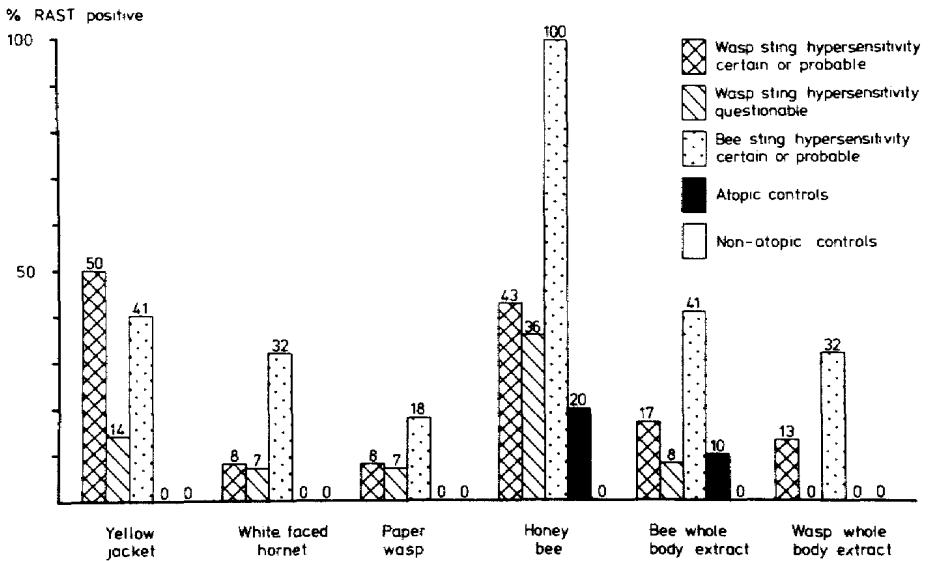


Fig. 1. RAST in wasp sting and bee sting hypersensitivity.

This means that 75% were RAST positive with at least one venom, if the serum was taken at an optimal interval after the exposure.

In RAST inhibition studies (Fig. 2) clear-cut inhibition of BWBE RAST was obtained by BWBE and by HB, whereas HB RAST was

only inhibited by HB but to no significant extent by BWBE. Ten patients with bee sting hypersensitivity without known allergic reactions to wasp stings but a positive RAST to both HB and YJ were investigated further (Fig. 3): In two of them absorption with HB

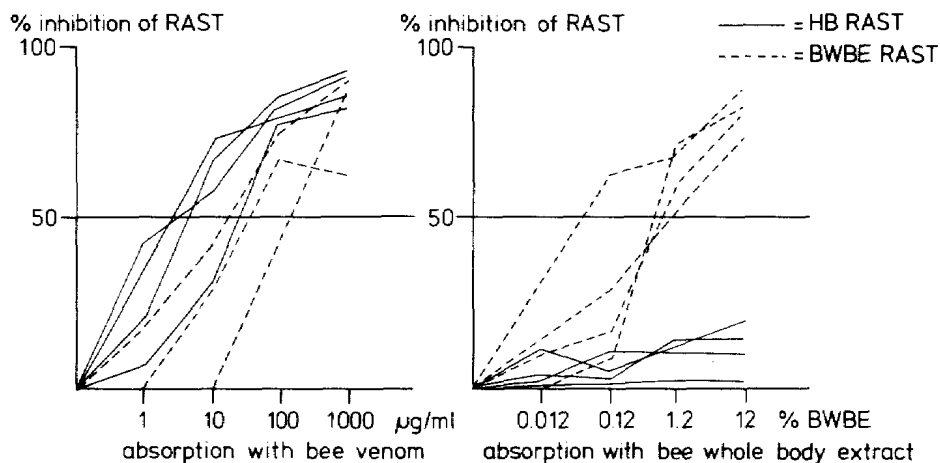


Fig. 2. Inhibition of RAST with bee venom (HB) and bee whole body extract (BWBE) in four patients with bee sting hypersensitivity.

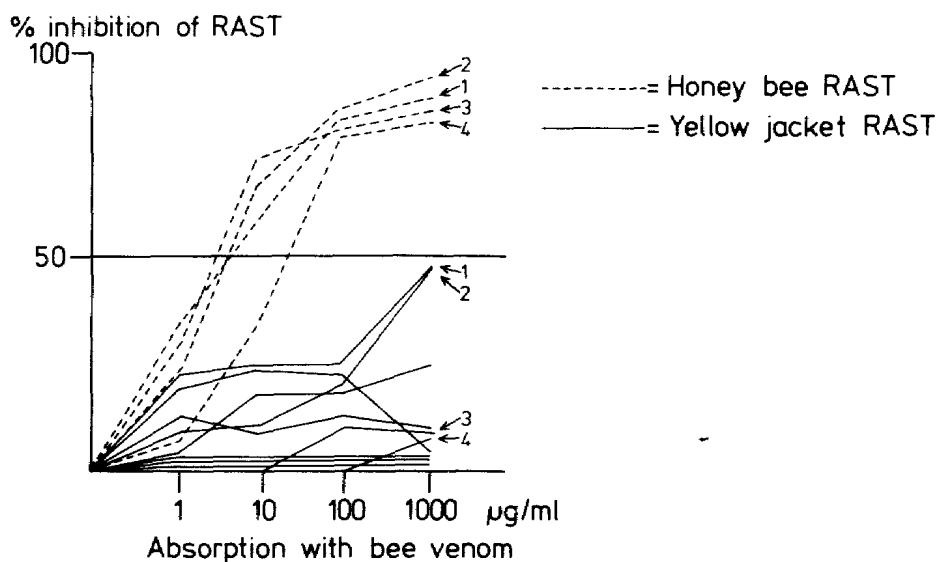


Fig. 3. Inhibition of RAST with bee venom (HB) in 10 patients with clinically pure bee sting hypersensitivity but serum IgE-antibodies to both bee venom and yellow jacket venom.

at a concentration of 1 mg/ml, caused 45% inhibition of the binding to the YJ discs, higher concentrations of HB resulted in no greater inhibition. No significant inhibition was observed in the remaining eight patients.

DISCUSSION

The results obtained with the YJ RAST discs in patients with suspected wasp hypersensitivity and in non-allergic controls show a fair

correlation with case history (Table 1): IgE-antibodies to yellow jacket were found considerably more frequently in patients with probable or convincing than in those with only questionable wasp sting hypersensitivity, and they were never detected in the control group of atopic and non-atopic blood donors. The results are, however, not as good as those previously reported (7) with HB RAST in HB-allergic patients. This could be explained by the fact that the YJ antigen originated from the American wasp *Vespa maculifrons*, a near relative of the European wasp, but not completely identical to it. On the other hand, HB hypersensitivity is much more frequent in Switzerland than wasp hypersensitivity and the HB is most easily identified, because its sting usually remains in the skin. Therefore misidentification is probably rarer in HB stings than in stings of other insects. This view is supported by the fact that seven patients with suspected wasp hypersensitivity were RAST negative with YJ and positive with HB. With either antigen there is a substantial number of patients who have no detectable serum IgE-antibodies. Possible explanations for this have been discussed previously (7). Neither this nor previous studies (7) have shown evidence of significant interference in the Phadebas RAST by "blocking factors" of serum like e.g. IgG antibodies.

Three of eight patients with anamnestic suspicion of hypersensitivity to wasp stings only and nine of 22 patients with anamnestically pure bee sting hypersensitivity were positive with both YJ and HB. Seven of the 22 patients with bee sting hypersensitivity also had specific IgE to WFH and four to PW without remembering stings by these insects. These observations suggest the existence of cross-reactions between the various *Hymenoptera* venoms. On the basis of inhibition studies of the Prausnitz-Küstner test Loveless & Fackler (5) assumed the existence of common as well as species-specific antigens in various *Hymenoptera* venoms. RAST inhibition studies, however, showed no significant cross-reactions between the YJ

and HB in most patients studied and conflicting results in two of them. These results are similar to those of Kern *et al.* (2) who, while using the histamine release assay, could not find cross-reactions between various vespid venoms, except for a modest association between two different hornet venoms. This in spite of the fact that leucocytes of most patients released histamine with three or four of the venoms tested, even when the patients were exposed to only one insect species. As all our patients were studied before initiation of immunotherapy, a sensitization due to the use of mixed insect extracts can be excluded. The hypothesis of Kern *et al.* (2) that IgE-antibodies are directed against multiple allergens in insect venoms of which only a few are cross-reacting, could explain the partial RAST inhibition observed in some of our patients. It could be confirmed by performing RAST inhibition studies with isolated venom antigens, for instance phospholipase A of both bee and wasp. Crossed immunoelectrophoretic and radioimmuno-electrophoretic methods could offer another approach to this problem.

On the basis of this hypothesis it is conceivable that patients with clinical suspicion of hypersensitivity to only one insect, but IgE-antibodies to several *Hymenoptera* venoms, could be treated effectively with the venom of the clinically offending species alone. It will, however, be safer to treat these patients with a mixture of all *Hymenoptera* venoms against which IgE-antibodies were found, because misidentification of stinging insects can only rarely be ruled out completely.

Venoms gave considerably better results than whole body extracts both in bee- and in wasp-hypersensitive patients. Absorption with BWBE caused no significant inhibition of the HB RAST results in all individual patients studied, while absorption with HB inhibited BWBE RAST results regularly. Light *et al.* (4) got similar results when using a pool of sera from patients with bee sting allergy. This indicates that IgE-antibodies

detected by the BWBE RAST technique are directed against bee venom constituents in the whole body extract. Together with the antibody studies during immunotherapy (3, 8, 10) it is further evidence in favour of the use of venom instead of whole body antigens for diagnostic and therapeutic purposes. Except for the honey bee, venom preparations are not commercially available at present, but will probably be so in a near future. Until then, diagnosis must be based mainly on clinical history, and hyposensitization therapy be carried out with the commercially available whole body extracts. The value of such a therapy is, however, controversial (3, 8, 10). Diagnostic procedures with whole body extracts as the antigen are thought to be unreliable.

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