

Skin and radioallergosorbent tests in patients with sensitivity to bee and wasp venom

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Summary

Intradermal (ID) and prick tests with bee or wasp venom (Pharmalgen) have been performed on 102 subjects with a history of adverse reactions to stings and forty-six control subjects giving no such history. Venom was diluted 100, 10 and 1 $\mu\text{g/ml}$ for prick testing and 10^{-2} , 10^{-3} and 10^{-4} $\mu\text{g/ml}$ for ID injections.

In forty-six control subjects all were tested with the highest concentration of prick testing solution (100 $\mu\text{g/ml}$), eight (17%) had positive reactions, a similar reaction rate to that reported in control subjects using 10^{-1} $\mu\text{g/ml}$ ID. In our 102 test subjects skin tests were therefore regarded as positive only if the reaction was elicited by 10 $\mu\text{g/ml}$ or less by prick test of 10^{-2} $\mu\text{g/ml}$ or less ID.

In general the results with skin prick tests and ID tests were comparable when the prick solution was 1000 times the concentration of that used for ID testing. ID tests were positive in thirteen with negative skin prick, seven of whom had detectable antibodies when tested by RAST. Conversely four with a positive skin prick test (two of whom were RAST positive) were considered negative on ID testing. As judged either by RAST or skin tests it appeared that sensitivity diminished with the time interval from the last sting ($P < 0.001$).

Introduction

While immunotherapy with bee and wasp venoms now has an established place in the treatment of hypersensitivity reactions to insect stings, it is still difficult to identify those patients who would be likely to develop severe reactions to further stings and therefore require treatment. Once immunotherapy is begun, there may be uncertainty about whether the patient will once again be at risk when the therapy is terminated.

In the present study an attempt has been made to analyse the clinical and immunological response of 102 patients who presented at an allergy clinic with a history of a severe reaction to a bee or wasp sting. In each case, the patient's symptoms were analysed, together with the results of skin prick tests, intradermal tests and RAST to the appropriate venom.

Table 1. Adverse reactions* to bee/wasp venom

Urticaria	68
Chest 'tightness'	38
Loss of consciousness	20
Angioedema	20
Vomiting	8
Abdominal colic	4
Chest pain	3
Total	102

* Most patients had more than one type of reaction. Of the twenty patients with angioedema, eighteen also developed urticaria (not listed separately).

Patients and methods

All patients were referred to the allergy clinic because they had a history of adverse reactions to bee or wasp sting (see Table 1) and were to be considered for desensitization. Intradermal tests were made with venom diluted in human serum albumin to 10^{-4} , 10^{-3} , 10^{-2} and 10^{-1} $\mu\text{g/ml}$ respectively, 0.05 ml of each dilution being injected. Prick tests were performed with more concentrated venom, at 1, 10 and 100 $\mu\text{g/ml}$ respectively. Human serum albumin was used both for ID and prick test controls. IgE antibody to bee and wasp venom was measured by radioallergosorbent test (RAST), as modified by Kemeny, Lessof and Trull (1980) using cyanogen bromide-activated (CNBr) cellulose filter paper discs.

Briefly, 0.1 ml of a 1/10 dilution of patient's serum in horse serum (HS) (Sera Lab, Guildford, England) was incubated with an antigen-coated disc (10 mg venom/g paper disc) for 18 hr at room temperature (25°C). Unbound immunoglobulin was washed off with phosphate-buffered saline (PBS), 0.05 M, pH 7.4 containing 0.05% Tween 20, using a constant flow washing device (Kemeny & West, 1981) and the disc incubated for 18 hr with 10 ng of I^{125} radio-labelled anti-IgE (kind gift from Professor K. Ishizaka) at room temperature. Unbound I^{125} anti-IgE was washed off as before and the residual radioactivity counted in an LKB ultragamma counter for 1 min. All samples were assayed in duplicate and results expressed as units by reference to standard curves prepared using dilutions of a pool of sera taken from bee or wasp venom allergic patients. The pooled reference sera contained a high titre of IgE antibody to bee or wasp venom and units of RAST are equal to the percentage counts bound in RAST at the various dilutions of the serum pool, which was arbitrarily assigned 1000 units/ml. Thus one unit of activity was taken as being equal to the binding of 100 μl of this reference serum pool at 1/1000 dilution. Results were considered significant if they were more than 3 s.d. above the mean of six sera taken from non-allergic laboratory staff.

Results

Eight (17%) of forty-six control subjects with no previous history of adverse reactions to bee or wasp stings were sensitive to prick tests using 100 $\mu\text{g/ml}$ of venom, the highest concentration. In view of this only those subjects reacting to venom diluted 10 $\mu\text{g/ml}$ or

Table 2. Minimum concentration of bee or wasp venom required to give positive skin reaction comparing ID injection with skin prick method

Prick tests ($\mu\text{g/ml}$)	Intradermal tests ($\mu\text{g/ml}$)			Total
	10^{-1}	10^{-2}	10^{-3} or 10^{-4}	
100	40*	11	2	53
10	4	21†	16†	41
1	0	3†	5†	8
Total	44	35	23	102

* Regarded as skin test negative by both methods.

† Regarded as skin test positive by both methods.

Four subjects sensitive to venom by prick test would have been regarded as ID negative. Conversely, thirteen subjects sensitive to ID venom were prick test negative.

less were regarded as skin sensitive. Similar studies in control subjects using intradermal (ID) tests have shown that about 10% react to a concentration of 10^{-1} $\mu\text{g/ml}$ (see below). Accordingly only those reacting to ID venom at 10^{-2} $\mu\text{g/ml}$ or less were regarded as being sensitive.

One hundred and two patients with a history of adverse reactions to stings were given both prick and ID skin tests using diluted bee or wasp venom. These methods were compared using 0.05 ml of venom at 10^{-1} , 10^{-2} , 10^{-3} or 10^{-4} $\mu\text{g/ml}$ for ID injection and concentrations of 100, 10 and 1 $\mu\text{g/ml}$ for skin prick (Table 2). The results were roughly comparable when the skin prick test solution was 1000 times more concentrated than that used for ID testing but some discrepancies occurred. Thirteen subjects who reacted to 10^{-2} , 10^{-3} or 10^{-4} $\mu\text{g/ml}$ ID, and were therefore considered sensitive to venom, failed to show a positive response to 10 $\mu\text{g/ml}$ by skin prick. Conversely four subjects reacting to 10 $\mu\text{g/ml}$ of venom by skin prick showed no response to ID venom. Comparison of these discrepancies with the presence of IgE antibodies in the serum, measured by RAST, provided an objective point of reference against which they could be judged. Of the thirteen subjects who had a positive ID skin test but a negative skin prick test, seven had a positive RAST, and two out of the four skin prick test-positive, ID skin test-negative subjects, had a positive RAST. These numbers are too small for any firm conclusions to be drawn, but the proportion of subjects with a positive RAST was the same whichever method was used to test skin reactivity.

A comparison was also made between the results of intradermal testing and RAST, expressed as U/ml (Fig. 1). Mean levels ($\pm$ s.e.m.) were 1.6 ± 0.51 U/ml in those reacting only to 10^{-1} $\mu\text{g/ml}$ ID who were not therefore considered sensitive to venom. Sensitive subjects reacting to 10^{-2} or to 10^{-3} and 10^{-4} $\mu\text{g/ml}$ by ID injections showed mean RAST scores ($\pm$ s.e.m.) of 4.6 ± 1.0 U/ml and 14.2 ± 1.9 U/ml, respectively.

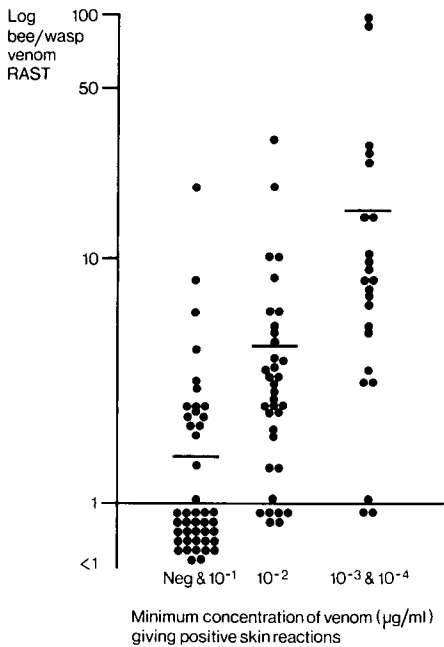


Fig. 1. Specific IgE antibody level *vs* sensitivity of ID test. Bars represent means of the RAST scores.

RAST results were also compared with the time which had elapsed since the last sting. The mean level of specific IgE was 10.2 ± 1.5 U/ml in those tested within 1 year of being stung, 3.3 ± 0.5 U/ml between 1 and 5 years of the sting and 1.3 ± 0.5 U/ml 5 years or more after the sting. These means were significantly different one from another ($P < 0.01$).

Both skin sensitivity, and the level of IgE antibody (as measured by RAST) diminished as a function of time elapsed since the last sting (Table 3). Thirty-three of the forty-seven (70%) who were tested within 1 year of being stung showed an

Table 3. Minimum concentration of bee or wasp venom giving a positive reaction compared with the time since the last sting

Time since last sting (years)	ID skin test threshold (µg/ml)				
	Total No.	Neg. or 10^{-1}	10^{-2}	10^{-3}	10^{-4}
<1	47	14 (30%)	20	10	3
1-5	34	15 (44%)	10	7	2
>5	21	15 (71%)	5	1	0
Total	102	44	35	18	5

Thirty percent of patients with a history of adverse reactions to venom were skin test negative when seen within 1 year of the sting.

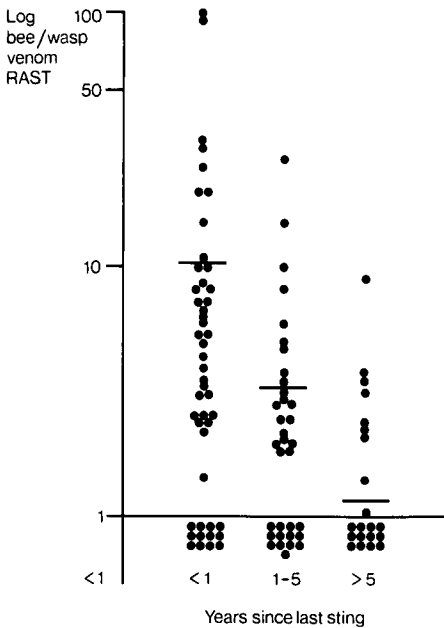


Fig. 2. Specific IgE antibody level *vs* time since last sting. Bars represent means of the RAST scores.

immediate skin reaction but only six of twenty-one (29%) stung 5 years or more prior to being tested, still retained their skin sensitivity. Likewise, thirty-five of the forty-seven (75%) who were last stung within 1 year, had a positive RAST, but only nine out of twenty-one (43%) last stung 5 or more years ago, still had detectable serum IgE antibodies (Fig. 2).

Discussion

Halonen *et al.* (1982) have demonstrated the close correlation between the skin prick tests and the level of specific IgE antibody to common environmental antigens, and we have found a further correlation between skin prick and intradermal tests when the solutions are freshly prepared. Björkander and Belin (1979), using the same concentrations of venom for prick testing as were used by us, found that out of fifty control subjects (with no history of adverse reactions to venom) twelve (24%) reacted to the highest concentration of 100 $\mu\text{g}/\text{ml}$. This compares favourably with our figure of 17%. Björkander's group also tested control subjects with intradermal preparations and found that 10% gave a weal and flare reaction with 10^{-1} $\mu\text{g}/\text{ml}$. A positive skin test to 10^{-1} $\mu\text{g}/\text{ml}$ ID or 100 μg by prick test in control subjects cannot thus be regarded as evidence of sensitivity although it is possible that some patients who are history positive, but who react only to ID venom at 1 or 10^{-1} $\mu\text{g}/\text{ml}$, are still at risk. We would regard a reaction to 10^{-2} $\mu\text{g}/\text{ml}$ or less ID or 10 $\mu\text{g}/\text{ml}$ or less by prick test as firm evidence of sensitivity. Since the more concentrated solutions which are used in prick tests have a long shelf life and are less prone to adsorption onto plastic or glass containers, prick testing has advantages of convenience in the identification of patients with antibody against bee or wasp venom. In addition, ID testing is uncomfortable for the patients, requires a higher level of skill to perform, and is more time consuming

than prick testing. Facilities to measure specific IgE antibody to the components of venom are not available to all centres. The acceptability of skin tests, whether ID or skin prick, therefore needs to be validated.

Our data suggest that skin prick and intradermal tests give comparable results when allowance is made for the difference in concentration which is needed and that both give comparable results with reference to RAST, used as an objective measure of sensitivity. Settupane and Chaffee (1979) reported that patients who had a history of systemic reactions to stings were less likely to suffer a worse reaction to a subsequent sting if it occurred within 2 weeks, or more than 5 years, following the last sting. Although, fatal reactions can occur in patients who have not been re-stung for 20 years (Youlten, personal communication), our data shows that both skin test reactivity and serum IgE antibodies decline with time elapsed since the last sting suggesting that, in general terms, the susceptibility of hypersensitive patients diminishes with time.

References

- BJÖRKANDER, J. & BELIN, L. (1979) Diagnostic skin testing in hymenoptera sensitivity. (Abstract) *Xth International Congress of Allergology, Jerusalem*, p. 27.
- HALONEN, M., BARBEE, R.A., LEBOWITZ, M.D. & BURROWS, B. (1982) An epidemiologic study of the interrelationships of total serum immunoglobulin E, allergy skin-test reactivity, and eosinophilia. *Journal of Allergy and Clinical Immunology*, **69**, 221.
- KEMENY, D.M., LESSOF, M.H. & TRULL, A.K. (1980) IgE and IgG antibodies to bee venom as measured by a modification of the RAST method. *Clinical Allergy*, **10**, 413.
- KEMENY, D.M. & WEST, F. (1982) Rapid washing of filter paper discs in a solid-phase radioimmunoassay with a constant flow washing device. *Journal of Immunological Methods*, **49**, 89.
- SETTIPANE, G.A. & CHAFFEE, F.H. (1979) Natural history of allergy to hymenoptera. *Clinical Allergy*, **9**, 385.

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