

Basophil histamine release in insect venom allergy

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The aim of the present study was to investigate venom-related and venom-non-related immunological reactions in patients stung by bee or wasp. Sixteen consecutive patients (7 with local and 9 with systemic reactions) were tested with skin tests, RAST and basophil histamine release (BHR) test immediately after the insect sting and 2, 4, and 16 weeks later. No test was useful immediately after the insect sting, the "anergic period". In agreement with earlier findings, the SPT was the only allergy test that showed statistically significant differences between patients with local and systemic reactions, although a great overlap was found. Release of histamine from basophils after incubation with anti-IgE also showed statistically significant differences between local and systemic reactions. Further studies are needed, especially measurement of BHR after incubation with anti-IgE before insect stings.

Key words: anti-IgE; basophil histamine release; intracutaneous test; RAST; skin prick test; systemic reactions; venom allergy.

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In the Scandinavian countries, insect stings regularly occur. Allergic reactions are most frequently seen after stings by wasp (*Vespula* species), and in some cases by honey bee (*Apis mellifera*).

After an insect sting most people only experience local swelling, itching and pain, which disappears within a few days. The most frequent abnormal manifestations are large local reactions (i.e. swelling with an approximate diameter > 10 cm) and systemic reactions (generalized reaction, including urticaria, anaphylactic shock, etc.).

Immunotherapy offers an effective protection against future IgE-mediated anaphylactic reactions (13). Results of skin testing and RAST have been described previously (1, 20), the diagnostic value has, however, been found to be

limited. Clinically, it is a problem to define the group of patients in whom future insect stings may lead to severe IgE-mediated systemic reactions and who should, therefore, be offered immunotherapy.

With special reference to local versus systemic reactions following a freshly experienced insect sting, the aim of the present study was to investigate the validity of the basophil histamine release (BHR) test by:

1. Elucidating whether venom allergen-related (BHR-all) and non-allergen-related (BHR-nonall) histamine releasing mechanisms offer results that are dependent on the clinical reaction following the insect sting; and
2. Determining the optimum time for immunological testing after an insect sting with respect to maximum reactivity of the tests

and to maximum ability of distinction between local and systemic reactions.

METHODS

Patients

In the course of one summer all consecutive patients between 20 and 65 years of age contacting the casualty ward, County Hospital, Roskilde, Denmark, less than 24 h after an insect sting were offered participation in the study. Twenty-two patients of either sex complied with these criteria. Seventeen of these patients were included in the investigation, the remaining did not want to participate due to lack of time and practical difficulties.

Five women and 11 men (mean age 39.4 years, range 20–64 years) participated in the investigation (one patient did not show up for controls). The material therefore consists of 4 patients with urticaria as the only systemic manifestation, 5 patients with more severe systemic reactions, 5 patients with a small local reaction (normal reaction), and 2 patients with large local reactions. Patients with local reactions served as control group.

Two patients were found to be allergic to both wasp and bee. Both claimed to have been stung by a wasp, but had earlier experienced bee stings. Twelve patients were allergic to wasp, which was in compliance with the history of 7 patients, 1 claimed to have been stung by a bee, and 4 patients claimed to have been stung by an unidentified insect. One patient was allergic to bee venom, which was in compliance with the history, and one patient, who claimed to have been stung by a wasp, was found not to be allergic to insect venom. Results are shown with reference to the type of insect by which the patients claimed to have been stung. For those stung by an unidentified insect, results giving the type of insect with the largest reactivity are used.

Investigations were carried out at the time of admittance to the casualty ward, and 2, 4, and 16 weeks later. Blood samples were drawn

at all occasions, skin testing after 2, 4, and 16 weeks.

Allergens

The raw material for insect venom allergen extracts was obtained from Vespa Lab. Inc., U.S.A. The extracts were purified to remove all low molecular substances (< 1000 Daltons), standardized by quantitative immune electrophoresis (QIE) and RAST-inhibition (9) and dissolved in 50% glycerol (Soluprick SQ®, ALK Inc., Copenhagen, Denmark) for skin tests. For the performance of the other tests, freeze-dried extract from the same standardized batch was used.

Basophil histamine release test

The method used was a fluorometric determination of histamine based on histamine release from whole blood basophils (24). Venous blood was anticoagulated with ethylenediaminetetraacetic acid (EDTA), gently shaken at room temperature, and preserved by adding an equal volume of cell culture medium (RPMI, Gipro Bio-Cult Ltd., Scotland). Immediately after, still at room temperature, the samples were taken to the laboratory (25) and analysed within 24 h. The blood cells were washed extensively as described in detail by Diamant & Patkar (4) and resuspended in buffer to a final volume of twice the original blood sample. One ml of washed blood corresponds to 0.5 ml of whole blood. Venom allergen was added to washed blood to a final allergen concentration of 6,000, 2,000, 200, 20, 2, and 0.2 ng/ml of whole blood, making a dose response curve. The samples were incubated at 37°C for 40 min. As a control of reactivity of the cells, rabbit anti-human IgE (14) was added to a final concentration of 500, 100, and 50 ng/ml of whole blood. Three samples without addition served as measures of spontaneous release of histamine, and 3 samples with an equal volume of 7% perchloric acid served as measure of total histamine content (ng/ml).

After incubation the samples were transferred to an ice bath for 10 min to stop the

reaction, and then centrifugated. The supernatants were transferred to Technicon cups and precipitated with an equal volume of 7% perchloric acid.

The release of histamine was estimated in the Technicon Autoanalyser System®. The results were calculated in per cent of the total histamine content, the spontaneous release being deducted. The results were graded (Table 1), classes 2 and 3 being regarded as positive, classes 0 and 1 as negative (27).

In adults, release of histamine from human basophils after incubation with anti-IgE have been shown to be independent of age (15).

Skin prick test

All patients underwent skin prick testing (SPT) with extracts of bee and wasp venom, respectively, in the concentrations of 30 µg/ml, 100 µg/ml, and 300 µg/ml. All tests were made in duplicate with the DHS lancet 1 mm (28), the results being read after 10 min. As positive control histamine dihydrochloride 1 mg/ml and 10 mg/ml were used, the glycerol diluent serving as negative control. A wheal with an approximate diameter of 3 mm or more was considered positive.

Intracutaneous test

Intracutaneous test (ICT) with extracts of bee and wasp venom in the concentrations of 0.1 µg/ml, 0.3 µg/ml, and 1.0 µg/ml were per-

formed on all patients. 0.05 ml of the allergen extract was injected intracutaneously. The results were read after 15 min; a wheal with an approximate diameter of 20 mm or more was regarded as positive (21).

RAST

The amount of allergen-specific IgE was measured by radioallergosorbent test (RAST) using paper-discs as the solid phase (5). The results were given in sorbent units (SU) compared to a reference serum from a patient with average allergy (= 100 SU/ml). RAST classes 3 and 4 were judged as a positive result, classes 0-2 as a negative result.

Concurrent allergies

Two patients (large local reaction and urticaria respectively) had typical atopic disease with asthma and allergic rhinitis. Several years ago, another 2 patients (small local and large local reaction, respectively) experienced isolated, short, inexplicable episodes of hives, but had no other signs of atopy. These 4 patients did not differ from the remaining patients with regard to venom-related and venom-non-related investigations. As the only one, the patient with asthma (urticaria following the insect sting) had a positive SPT (28) with one of the 10 most commonly occurring inhalant allergens.

Statistics

Comparison between groups was made non-parametrically by the Mann-Whitney test, 5% being the level of significance.

RESULTS

Two patients (large local and systemic reaction, respectively) were found not to react to anti-IgE with the BHR. In the remaining 14 patients, 3 of 6 patients with local reactions and 4 of 8 patients with systemic reactions offered positive results with the BHR-all test within 24 h of the

Table 1

Basophil histamine release test (incubation with venom allergen)

BHR-class	Histamine release %*	Venom concentration /ml whole blood
0 (negative)	0-8	all
1 (doubtful)	9-19	all
2 (positive)	≥ 20	> 200 ng/ml
3 (strong positive)	≥ 20	≤ 200 ng/ml

*Ratio between: released histamine after allergen incubation, and maximum released histamine after cell lysis by perchloric acid (4).

BHR-all class

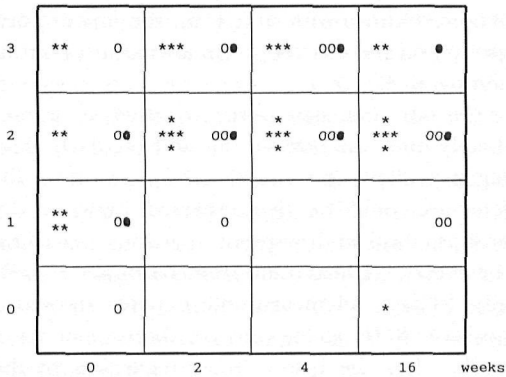


Fig. 1. Basophil histamine release test (BHR-all): BHR-classes in patients with systemic (*), large local (●), and small local (○) reactions following an insect sting.

ng histamine/ml whole blood

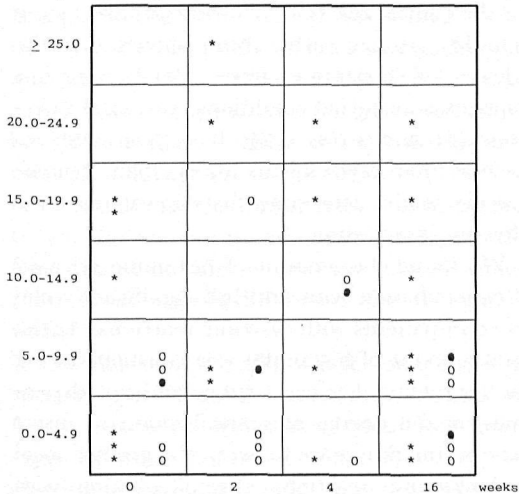


Fig. 2. Basophil histamine release test (BHR-nonall): Release of histamine (ng) after anti-IgE (500 ng/ml whole blood) in patients with systemic (*), large local (●), and small local (○) reactions following an insect sting.

insect sting. A significantly increased reactivity was found 4 weeks later, when all 6 patients with local and all 8 patients with systemic reactions offered positive results with the BHR-all test, $P < 0.05$ (Fig. 1). However, no significant difference between patients with local or systemic reactions was found in any of the four BHR-all tests (Fig. 1).

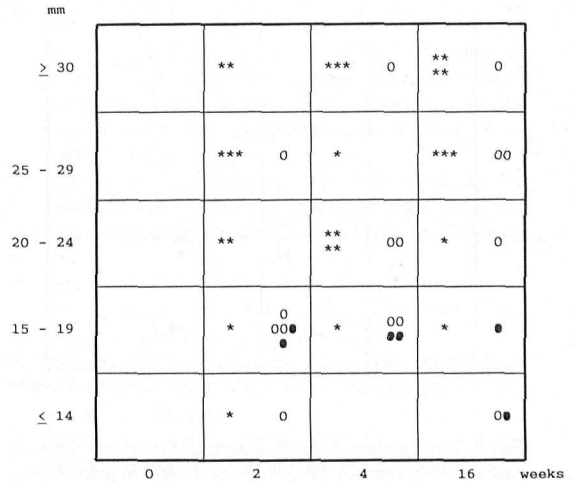


Fig. 3. Skin prick test (300 µg/ml): Wheal size (mm) in patients with systemic (*), large local (●), and small local (○) reactions following an insect sting.

After cell lysis with perchloric acid, measurement of the total histamine content in the blood samples showed equal amounts in the clinical groups, and no change during time was observed. Since this baseline histamine content was comparable in all groups, the amount of histamine released after incubation with anti-IgE was measured, showing significant differences between the groups' local and systemic reactions 16 weeks after the insect sting. Results using 500 ng anti-IgE/ml whole blood are shown in Fig. 2. We found no significant changes in reactivity after incubation with anti-IgE during the first 16 weeks. Due to lack of results, Fig. 2 is not completed for the 2 and 4 weeks after the insect sting.

SPT with extracts of insect venom in the concentrations 100 µg/ml and 300 µg/ml were equally good, both showing statistically significant differences between the groups with local and systemic reactions 2 and 4 weeks after the insect sting (Fig. 3), though considerable overlap was found. None of the concentrations used in the ICT (Fig. 4), or the RAST (Fig. 5) showed significant differences between the groups' local and systemic reactions at any time after the insect sting ($P > 0.10$).

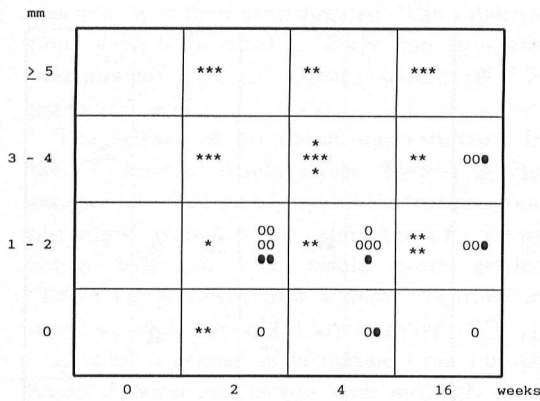


Fig. 4. Intracutaneous test (1.0 µg/ml): Wheal size (mm) in patients with systemic (*), large local (●), and small local (○) reactions following an insect sting.

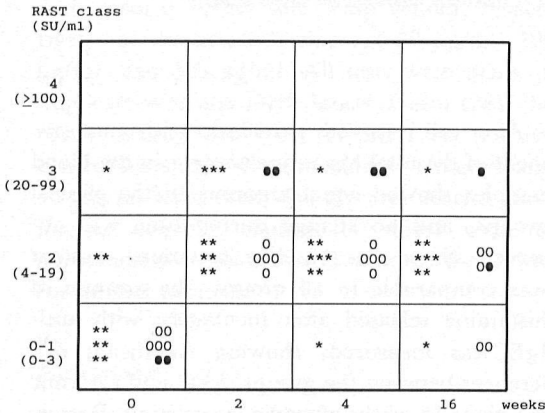


Fig. 5. RAST: Class and SU (in brackets) for patients with systemic (*), large local (●), and small local (○) reactions following an insect sting.

group. As the degree of sensitization to insect venom declines with time (16), the investigated group and the control group are not necessarily comparable.

All our patients were investigated immediately after the insect sting and regularly during the following 4 months. No significant differences could be demonstrated between the groups' local and systemic reactions regarding the release of histamine from basophils (BHR-all). In fact, all measurable patients showed a positive BHR-all to one or both venoms 4 weeks after the insect sting, regardless of the clinical reaction.

There are reports of patients experiencing systemic reactions without detectable venom-specific IgE (2). Previously, some investigators have explored whether mechanisms other than strictly allergen-IgE mediated are responsible for the clinical reaction following an insect sting (11, 18). It has earlier been shown that the release of histamine from blood basophils depends on external conditions, e.g. viral infections (8) and stress (22). It is reasonable to assume that mechanisms other than venom-specific might determine the clinical reactions after an insect sting.

We found the amount of histamine released after incubation with anti-IgE significantly elevated in patients with systemic reactions. As the total amount of histamine was constant during the time after the insect sting, and not dependent on the degree of sensitization to insect venom, the difference between the groups' local and systemic reactions after incubation with anti-IgE must depend on other factors. Possible explanations are the stability of the cell membrane of the basophils (10), the degree of binding of IgE to the cell surface, or other factors.

DISCUSSION

1. Basophil histamine release test

Previous investigations generally find the BHR comparable with the other tests available (6, 7, 17, 26); however, some find skin tests superior (23). In general, the control group had not been stung for many years in contrast to the investigated group or information is missing as to the time since the last insect sting in the control

2. Relation to other tests

The skin tests, especially the SPT 100 µg/ml and 300 µg/ml, were more valuable than the RAST in distinguishing between local and systemic reactions, which is in agreement with previous findings (19). The BHR-all test did not add further information. SPT did not show any significant changes during the first 16

weeks after the insect sting. In both the local and the systemic groups, the results of the ICT showed a significant increase in the period 2 to 16 weeks after the sting, and RAST a significant increase from immediately after the insect sting compared to all the following investigations. Our results therefore confirm the thesis of the "anergic period" during the first weeks following an insect sting (9), since all tests showed very little reactivity immediately after the insect sting, and both ICT, RAST, and BHR values increased significantly during the following weeks.

3. The concept of basophil releasability

The differences between the clinical reaction and the results of immunological investigations could, at least in part, be due to differences in basophil releasability (3). It has already been shown that releasability differs between children and adults (15). Whereas BHR can be measured direct *in vitro*, overall IgE production, effects of mediators, hormonal and dietary factors, all influence the *in vivo* response (12). The cellular basis for the higher release of histamine by anti-IgE in the systemic reactions than in the local, could be due to the same factors which result in the marked differences after incubation with D₂O.

In conclusion, the SPT was the only allergen-specific test showing significant differences between patients with systemic and local reactions following an insect sting. However, no discriminative values could be determined due to overlap between the groups. Giving only a few positive reactions immediately after the sting ("anergic period") the ICT, RAST, and BHR-all significantly increased during the first 16 weeks of follow-up. Venom-non-specific histamine release showed significantly increased release in patients with systemic reactions after an insect sting. No test, alone or in conjunction, could distinguish between local and systemic reactions, and both *in vivo* and *in vitro* tests should be performed. The optimum time for performing tests are, according to our results, approximately 4 weeks after an insect sting.

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