

Serological investigations in hymenoptera sting allergy: IgE and haemagglutinating antibodies against bee venom in patients with bee sting allergy, bee keepers and non-allergic blood donors

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Summary

Specific IgE-antibodies and haemagglutinins to bee venom were determined in fifty-five bee sting allergic patients, fifty-seven bee keepers and fifty-two blood donors without evident allergy to hymenoptera stings. IgE-antibodies were detected by RAST in 70% of allergic patients, 40% of bee keepers and 12% of blood donors.

Most bee keepers with detectable IgE-antibodies to bee venom recorded severe local or even general reactions to bee stings. Most blood donors with detectable specific IgE had been stung by hymenoptera in the past.

High titres of haemagglutinating antibodies against phospholipase A were found in most bee keepers, occasionally in bee sting allergic patients but only rarely in blood donors.

The determination of specific IgE-antibodies to insect venoms by RAST seems to be a valuable method for the diagnosis of hymenoptera sting allergy.

Introduction

Until recently the diagnosis of allergic reactions to insect stings was primarily based on clinical history (Hoigné, 1965; Hoigné *et al.*, 1974). Skin tests with whole body extracts of insects, though widely used, are of little diagnostic value (Bernton & Brown, 1965; Schwartz, 1965). Besides they may sometimes cause serious generalized reactions. The first reliable *in vitro* test was described in 1974 by Lichtenstein, Valentine & Sobotka, who demonstrated IgE-mediated histamine release from the leucocytes of bee sting sensitive patients incubated with bee venom or phospholipase A, its major antigen. The test, however, is too laborious for routine clinical use. For this reason other authors (Reisman *et al.*, 1974; Yunginger *et al.*, 1975) like ourselves prefer another technique, the radioallergosorbent test (RAST), developed by Wide, Bennich & Johansson (1967). The aim of this study was to evaluate RAST as a diagnostic procedure in bee sting allergy. With regard to a possible significance in judging the success of hyposensitization therapy, haemagglutinating antibodies have also been determined.

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Materials and methods

Sera were collected from patients with sensitivity to honey-bee stings, bee keepers and blood donors. From the sixty-three selected patients, fifty-five had experienced generalized reactions and eight a severe local reaction following the stings. Patients with symptoms manifesting after 30 min exposure were excluded from this study. The allergic patients were divided into five groups (Table 1) according to the severity of the reaction following Mueller (1959) with some minor modifications.

Table 1. Classification of allergic reactions to hymenoptera stings (after Mueller, 1959).

Grade of reaction	Number of patients	Signs and symptoms
0 Severe local reaction	8	Diameter more than 5 cm, duration more than 24 hr
I Slight general reaction	10	Generalized urticaria, itching, malaise and anxiety
II General reaction	19	Any of the above plus two of following: angioneurotic oedema, dizziness, constriction of chest, abdominal pain, nausea, vomiting, diarrhoea
III Severe general reaction	14	Any of the above plus two of following: dyspnoea, dysphagia, hoarseness or thickened speech, confusion, feeling of impending disaster
IV Shock reaction	12	Any of the above plus two of following: cyanosis, fall in blood pressure, collapse, incontinence, unconsciousness

Sera from fifty-seven active bee keepers were collected during meetings of bee keeper associations in the canton of Bern, Switzerland. Forty-one bee keepers had kept bees for more than 1 year (long-standing) and sixteen had been involved in their hobby for less than 1 year (new-coming).

Sera of fifty-two blood donors were obtained from the blood transfusion centre of Bern. Donors with general or severe local reactions to hymenoptera stings were excluded.

Antigens

Bee venom was obtained from Sigma chemicals, U.S.A. and Fa Mack Nachf. Illertissen, BRD, bee venom phospholipase A by Fa Mack Nachf. Illertissen.

Radio-allergosorbent test (RAST)

BrCN-activated paper discs coupled with bee venom were made available through the courtesy of Dr Lars Yman (Research Department, Pharmacia Diagnostics AB, Uppsala, Sweden). The other reagents used were of the Phadebas RAST® (Pharmacia Diagnostics AB, Uppsala, Sweden) and the technical procedure was the same as in the commercially available test. Briefly, the antigen-coated paper discs were incubated for 3 hr with test sera and then washed three times with saline followed by an overnight incubation with isotope labelled anti-IgE. After another three washings with saline the radioactivity of the discs was measured in a gamma-counter. The classification into

RAST-classes 0–4 according to the number of counts registered was based on the Phadebas RAST reference system (references A, B, C, D). All determinations were done in duplicate and repeated if deviation of single values from the mean value was greater than $\pm 10\%$.

Haemagglutination

Whole bee venom was unsuitable for this procedure because of the strong haemolytic activity of one of its components, mellitin (Habermann, 1974). Tanned human red cells, O Rhesus negative, were therefore coated with phospholipase A, the major antigen of bee venom (Sobotka *et al.*, 1976) according to the technique described by Arbesman *et al.* (1960). In order to destroy reaginic activity sera were heated at 56°C for 1 hr before haemagglutination. The results are given in titres of the serum dilution at which the end point of haemagglutination occurred (Spiess, 1977).

Results

RAST

Bee venom specific IgE-antibodies (RAST score 1–4) were found in 70% of patients with generalized reactions, 50% of patients with severe local reactions, 40% of bee keepers and 12% of blood donors (Fig. 1). The differences between patients, bee

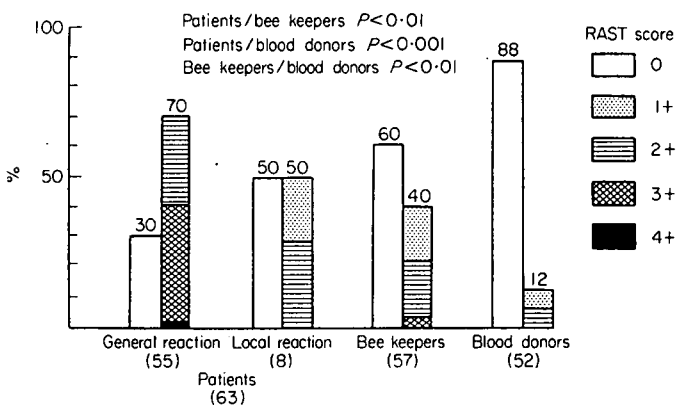


Fig. 1. IgE antibodies against bee venom in patients with bee sting allergy, bee keepers and blood donors.

keepers and blood donors were statistically significant. In patients with clinical manifestations of grade 0, RAST scores tended to be lower than in those with grade I to IV manifestations. In these latter cases there was no clear-cut correlation between severity of the symptoms and RAST class (Fig. 2). On the other hand, the time interval between last exposure and date of blood sample proved to be important. In patients with generalized reactions tested within 6 months after the last sting, 86% were RAST class 1–4. If more than 6 months had elapsed only 52% of patients tested were RAST positive (Fig. 3). Three patients were investigated immediately following exposure and at approximately weekly intervals thereafter. All showed an increase in specific IgE-antibodies during the first 2 weeks.

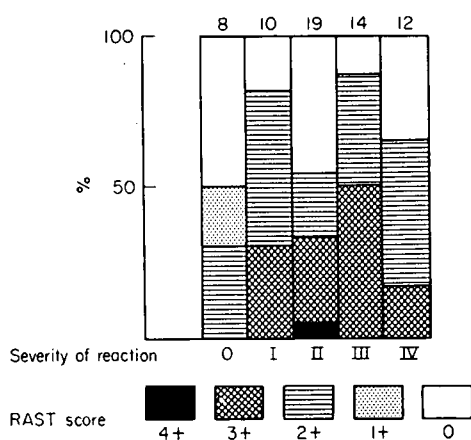


Fig. 2. Correlation between severity of reaction and RAST score.

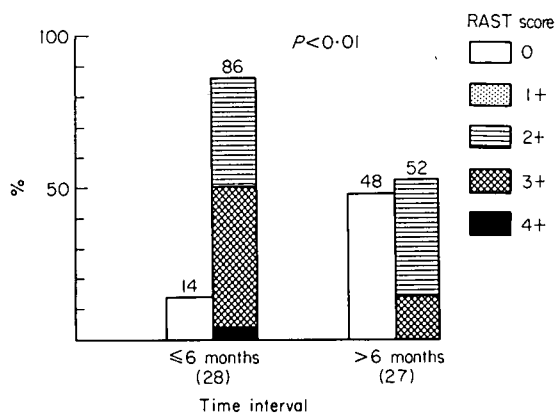


Fig. 3. Effect of time interval between last sting and date of blood sample on RAST score in fifty-five patients with general allergic reaction.

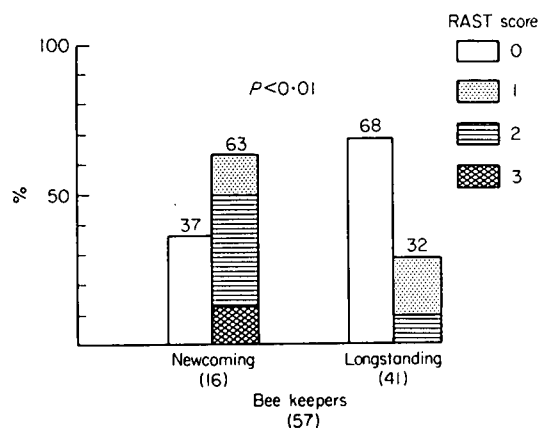


Fig. 4. IgE antibodies against bee venom in new-coming and long-standing bee keepers.

Of forty-one long-standing bee keepers 32% had detectable specific IgE and most of them were in RAST class 1 but 63% of the sixteen new-comers were shown to have specific IgE, most of them in RAST class 2 and 3 (Fig. 4). All bee keepers within RAST class 1-4 had experienced significant or severe local reactions and four even slight general reactions. Among long-standing bee keepers with no detectable specific IgE, three remembered having had a severe local reaction and four experienced a general reaction when they started bee keeping years ago. Bee keepers with high sting frequency tended to have lower IgE-antibody levels than those with low sting frequency (Fig. 5).

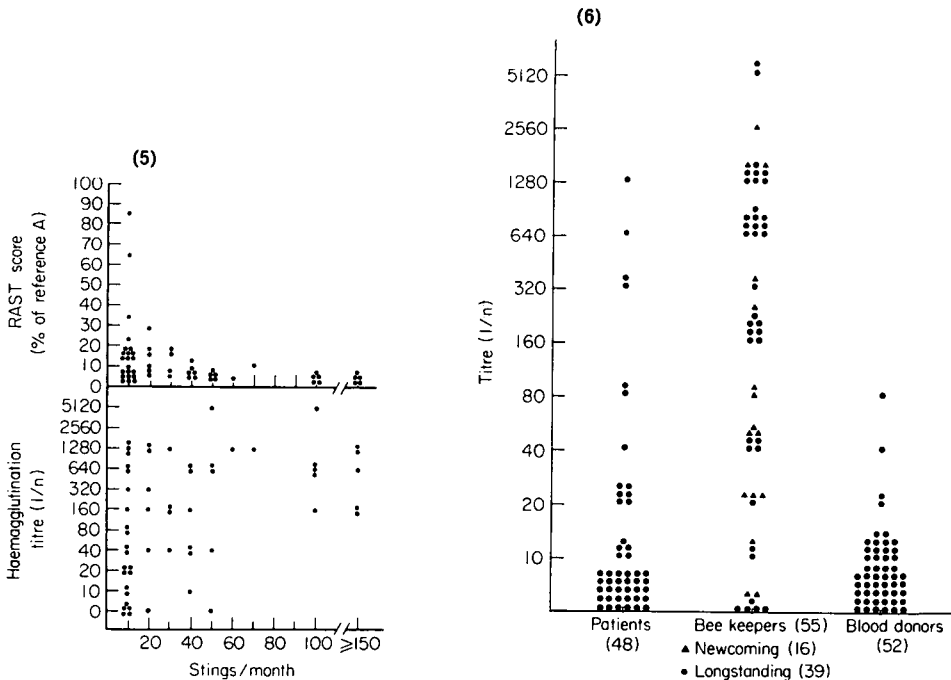


Fig. 5. Correlation between frequency of bee stings, specific IgE-antibodies and haemagglutinins in fifty-five bee keepers.

Fig. 6. Haemagglutinating antibodies against phospholipase A in bee sting allergic patients, bee keepers and blood donors.

All six blood donors in RAST class 1 and 2 had a personal history of atopic disease. Four of them remembered having been stung by hymenoptera within the last years followed by significant, though not severe local reactions. One further case could not recall any hymenoptera stings but reported severe local reactions following fly stings. The sixth did not remember any adverse reactions to insect stings. The two last mentioned blood donors without known exposure to hymenoptera stings were found to be RAST class 1.

Haemagglutination antibodies

Figure 6 shows that haemagglutinating antibodies were mainly found in bee keepers, less often in allergic patients and only rarely in blood donors. The difference between each group is statistically significant. The highest titres found were: 1/5120 in bee

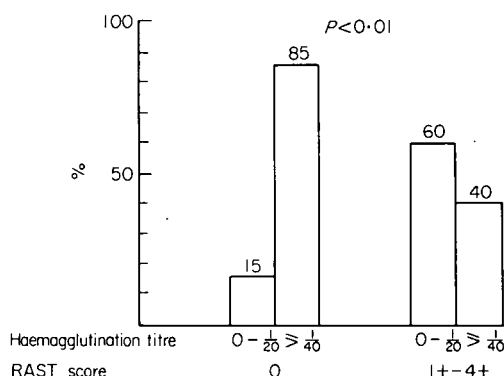


Fig. 7. Correlation between specific IgE-antibodies and haemagglutinins in bee keepers.

keepers, 1/1280 in patients (in a patient without detectable specific IgE) and 1/80 in blood donors. New-coming bee keepers tended to have lower titres than long-standing and those with a high sting frequency showed higher titres than those with a low one (Fig. 5). An inverse correlation between RAST-score and haemagglutination titre was observed (Fig. 7).

Discussion

Bee venom specific IgE antibodies were detected by RAST in a high proportion of bee sting allergic patients but only in a few blood donors with no generalized reactions and only two of the controls without significant local reaction. These results are in good agreement with those of other authors (Reisman *et al.*, 1975; Yunginger *et al.*, 1975). IgE-antibodies were detected in 50% of patients exhibiting severe local reactions suggesting that these may also be mediated by IgE-antibodies. This hypothesis can be supported by the clinical observation that the first general reaction is often preceded by a severe local reaction.

There was no correlation between the severity of a general reaction and the RAST class. The time interval between venepuncture and the last allergic reaction, however, was important. The observation of a rise in specific IgE during the first 2 weeks after exposure points to antibody consumption by the antigen or to stimulation of IgE-production following the sting. The lower incidence of detectable specific IgE after more than 6 months following exposure suggests a steady decline with increasing time interval. Nevertheless, IgE-antibodies may still be detected years after the last exposure. One of our patients had a high RAST score 11 years after the last exposure. Similar observations have been made in patients with penicillin allergy (Kraft *et al.*, 1977).

Beside this time factor there are some other possible explanations for a failure to detect specific IgE-antibodies to bee venom in some of our patients:

(1) The patient may have misidentified the stinging insect. It might have been a wasp or hornet. Cross-reactions between venoms from various hymenoptera species may occur but are not necessarily present in every case.

(2) Spontaneous hyposensitization following the last sting could have occurred. This has been observed by the authors in several patients whose allergic reactions gradually diminished over years or even suddenly disappeared. The occurrence of such spontaneous hyposensitization is also documented in a large scale study of the Insect

Allergy Committee of the American Academy of Allergy (1965). It seems to be rare in patients with anaphylactic shock (Hoigné, 1965).

(3) Specific IgE-antibodies may have been present in tissue in sufficient amounts to elicit an allergic reaction but nevertheless not be detectable in serum.

(4) Finally, some of the allergic reactions could have been mediated by other than IgE-antibodies (Reid, 1970).

In four blood donors with detectable specific IgE-antibodies and recorded to tolerate hymenoptera stings in their past, sensitization following their last sting cannot be excluded. In the one with strong cutaneous reactions to fly stings, antibodies to antigens related to bee venom constituents may have been present and caused a cross-reaction. Thus in only one blood donor with RAST class 1 no reaction at all to insect stings was recorded. This atopic person might have been sensitized by ingestion of bee allergens with honey or by inhalation of ubiquitous airborne bee antigens.

The high proportion of bee keepers with RAST class 1–3 is somewhat surprising. Most of the people heavily exposed to bee venom seem to become sensitized initially with strong local or even general reactions as the clinical equivalent. With continued exposure the concentration of IgE-antibody gradually decreases, whereas haemagglutinin titre which probably represents blocking antibodies (Busse *et al.*, 1975) increases in a direct relationship to sting frequency. This, together with the observation that haemagglutinins also increase under specific hyposensitization therapy, indicates that they are a good parameter for the degree of exposure with the antigen. The inverse correlation between IgE and haemagglutinating antibodies could be interpreted as evidence for an indirect protective effect of haemagglutinins by interference with IgE-formation. Recent studies (Lesso, Sobotka & Lichtenstein, 1976) demonstrated successful protection of bee sting allergic patients by passive immunization with serum of a bee keeper suggesting also a direct protective effect of blocking antibodies. The good correlation between haemagglutinins and blocking antibodies in the histamine release assay observed by others (Busse *et al.*, 1975) also supports this view. In our material there were, however, several bee keepers within RAST class 2 and even 3 with no general or severe local reaction in spite of low titres of haemagglutinins. Thus other mechanisms of immunological protection may also play a role.

To conclude, the authors consider that RAST is a valuable *in vitro* method for the diagnosis of hymenoptera sting allergy, especially if performed between 2 weeks and 6 months after the last sting. It should in the future replace unreliable and dangerous skin testing procedures in this type of allergy. Titres of haemagglutinating antibodies against phospholipase A are a good parameter of the degree of exposure to bee venom and may help in judging the success of hyposensitization therapy.

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