

The use of the radioallergosorbent test in the diagnosis of Hymenoptera anaphylaxis

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Summary

IgE antibody levels to Hymenoptera (honey bee, hornet, wasp or yellow jacket) venom or venom sac were measured in the serum of ninety-six patients with a history of immediate hypersensitivity reactions to these insect stings. Normal levels of IgE antibody were found in forty-four of these patients tested. The severity of the systemic reaction was similar in those patients with normal and elevated IgE antibody levels, and the interval from the systemic reaction to antibody determination was also similar. In twelve patients with a definite reaction to honey bee venom, the IgE antibody was elevated in all and correlated with a positive venom skin test in nine out of the twelve. It is not possible to determine if the other patients with normal IgE antibody and a systemic reaction represent a false negative RAST value, loss of sensitivity to the Hymenoptera venom, or a lack of RAST sensitivity with some venom or venom sac antigens. Without the ready availability of venom skin tests to all Hymenoptera antigens or other *in vitro* tests, a definite assessment of insect anaphylaxis remains in doubt for the patient and physician.

Introduction

Severe anaphylaxis can follow insect stings in sensitized individuals. It has been estimated that immediate hypersensitivity to stings from insects of the Hymenoptera order are found in about 0.5% of the general population. The clinical history is usually sufficiently characteristic to allow the physician to identify patients who have experienced these reactions. However, in many cases, identification of the offending stinging insect by the patient is most difficult. Skin testing with whole insect body extracts has been of questionable reliability in differentiating the allergic from non-allergic individual (Barr, 1972; Schwartz, 1965). Sobotka *et al.* (1974) demonstrated that in the majority of patients with anaphylactic sensitivity to insect stings, IgE antibodies were

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present to mediate histamine release from basophils. Differentiation of the allergic from normal subjects by leucocyte histamine release was possible only when venom, not whole body extract, was employed. Hunt *et al.* (1976) found a positive correlation between venom skin test sensitivity and histamine release from basophils. The same investigators showed venom superior to whole body extracts in skin testing to differentiate insect-allergic subjects from controls. Unfortunately, aside from honey bee, venoms from other Hymenoptera are not commercially available for diagnostic skin testing.

Identification of specific IgE antibodies by the radioallergosorbent test (RAST) has shown good clinical and skin test correlation (Yunginger & Gleich, 1975). In contrast to venom skin test reagents, quantification of serum IgE to several Hymenoptera venoms or venom sacs is commercially available by RAST. In the following study we measured IgE antibody to Hymenoptera venom or venom sacs in ninety-six patients seen in consultation for possible Hymenoptera sensitivity.

Materials and methods

Patient selection

Serum samples were obtained from patients seen in consultation at the University of Wisconsin Allergy Clinic for possible Hymenoptera hypersensitivity. The serum samples were collected at the time of consultation during the years 1975–76. All ninety-six patients studied had a clinical history of an immediate systemic reaction following a Hymenoptera sting. The systemic symptoms ranged from mild urticaria to severe angioedema and loss of consciousness. The patients ranged in age from 4 to 70 years old. Not included in this survey were patients who had received whole body immunotherapy. All serum collection was done at least 1 month following the most recent reaction.

Skin testing

Intradermal skin tests were performed with honey bee venom (Center Laboratories, Port Washington, New York). An initial 0.02 ml of 1 : 1,000,000 (w : v) extract was injected. If no response occurred at 15 min, the second concentration (0.02 ml of 1 : 100,000) was injected. A positive response was considered to be a 6–8 mm weal 15 min following the injection. No skin test response was elicited in non-allergic subjects when either the 1 : 1,000,000 or 1 : 100,000 venom extract was used.

RAST reagents

Venom sacs from hornets, wasps and yellow jackets (Davis Insect Venoms, Oshawa, Ontario, Canada) were homogenized in 0.2 M H_3BO_3 plus 0.04 M NaOH and 0.16 M NaCl (borate-saline, pH 8.0) at a concentration of 40–50 mg/ml for 6.5 min at 15,000 rev/min in a model 60K Virtis homogenizer (The Virtis Company Inc., Gardiner, New York). Particulate matter was removed by centrifugation, and the protein contents of the supernatant fluids were measured by the biuret reaction using human serum albumin as the standard (Kabut & Meger, 1967). Honey bee venom (Champlain Valley Apiaries, Middlebury, Vermont, or Sigma Chemical Co., Grade I, St Louis, Missouri) was prepared at 25 mg/ml in borate-saline. Solid phase RAST reagents were prepared by reacting approximately 100 mg venom sac protein or 150 mg honey

bee venom per g of cyanogen bromide-activated microcrystalline cellulose (Gleich & Yunginger, 1976).

RAST procedure

The RAST was performed in the Allergic Diseases Research Laboratory at Mayo Clinic, as previously reported (Gleich & Yunginger, 1976). In each assay a non-allergic control system was also tested, and results were expressed as a percentage of the total radioactive counts bound by this negative control serum. Values below 150% of control were considered negative; values greater than 200% of control were considered positive. Values between 150–200% were termed borderline.

Results

No elevation in IgE antibody was found in forty-four of the patients tested (see Table 1). Elevated levels of venom IgE antibody were found in forty-two patients and most frequently this was to honey bee venom (see Table 2). This may not reflect the true frequency with which insects produced systemic reactions but the fact that seven of these patients were bee-keepers. Elevated serum IgE antibody levels to honey bee

Table 1. Frequency of elevated IgE antibody to Hymenoptera venom or venom sac as detected by the radioallergosorbent test

RAST result	Frequency* (No. of patients/percentage total)
Negative	44/46
Borderline	10/10
Positive	42/44
Total	96/100

* The frequency indicates the number of patients with elevated IgE antibody levels to at least one insect.

Table 2. Incidence of Hymenoptera insect identified by RAST in forty-two patients with elevated IgE antibodies

Insect	Incidence*
Honey bee	26
Yellow jacket	13
Wasp	10
Hornet	8
Bumblebee	1

* Ten patients had elevated levels of IgE antibody to more than one insect.

components have been noted in previous studies involving bee-keepers and their family members (Yunginger *et al.*, 1975).

In the majority of the patients tested, the serum sample was obtained within 2 years of their reaction (see Table 3). However, patients who had experienced their last reaction over 10 years ago were more likely to have normal rather than increased IgE antibody concentrations to venom (20% to 10%, respectively).

An immediate systemic reaction was experienced by all patients studied. Comparison of symptoms during this reaction was similar in patients with normal as well as those with elevated IgE antibody levels (Table 4.)

Twelve patients were clearly able to identify the honey bee as the culprit insect having provoked the anaphylactic reaction. These individuals were bee-keepers, family members of bee-keepers, or found a stinger at the sting site. None of these twelve patients had normal honey bee venom IgE antibody levels (see Table 5). Immediate positive skin test reactions were found in nine of these twelve patients.

Table 3. Time interval of the most recent systemic reaction to a Hymenoptera sting and the determination by RAST of the IgE antibody to Hymenoptera venom or venom sac

Time interval (years)	Normal IgE (negative RAST)*	Elevated IgE (positive RAST)*
0.0-0.5	16(36)	13(31)
0.5-1.0	7(16)	9(21)
1.0-2.0	3(7)	4(10)
2.0-3.0	4(9)	1(2)
3.0-5.0	1(2)	5(12)
5.0-10	4(9)	6(14)
Over 10	9(20)	4(10)
Total	44	42

* Expressed as number (percentage of total in parentheses).

Table 4. Comparison of symptoms following a Hymenoptera sting in patients with normal (negative) or elevated (positive) IgE antibodies as determined by RAST

Symptom	Negative (forty-four patients)*	Positive (forty-two patients)*
Pruritis	26(59)	26(62)
Hives	27(61)	29(69)
Angioedema	26(59)	25(60)
Difficulty breathing	24(55)	21(50)
Wheezing	8(18)	9(21)
Faintness	16(36)	14(33)
Vomiting	4(9)	5(12)
Loss of consciousness	5(11)	2(5)

* Number of patients with this symptom (percentage of total in parentheses).

Table 5. The frequency of IgE anti-venom antibody to bee venom in twelve patients with bee sting anaphylaxis and comparison to bee venom skin test results

RAST	Skin test		Total
	Positive	Negative	
Positive	8	1	9
Borderline	1	2	3
Negative	0	0	0
Total	9	3	12

Discussion

With the availability of IgE antiserum, Wide, Bennich & Johansson (1967) developed the *in vitro* test for the measurement of serum IgE antibodies. The radioallergosorbent test has proved valuable in the identification of IgE antibodies to a wide variety of pollens (Aas & Joansson, 1971), house dust and house mites (Stenius & Wide, 1969), fish (Aas & Johansson, 1971) and moulds (Yunginger, Roberts & Gleich, 1976). Correlation of RAST results with mucosal membrane challenges has been good (Berg, Bennich & Johansson, 1971). Good correlation has also been found in highly sensitive ragweed patients between IgE antibody, as determined by RAST, and both ragweed antigen-induced histamine release from leucocytes and symptom indices (Lichtenstein, Ishizaka & Norman, 1973). A significant relationship also exists between ragweed antibodies, as determined by RAST, and both symptom scores and end-point skin test titration in ragweed-sensitive patients (Yunginger & Gleich, 1975). These observations lend considerable support as to the sensitivity and specificity of the RAST for determination of IgE antibodies.

Reisman, Wypych & Arbesman (1975) employed RAST methods to detect IgE antibodies in 109 patients with insect hypersensitivity reactions. In only eighteen patients with histories of systemic reactions did these authors fail to detect elevated IgE levels to Hymenoptera venom. There are several important areas in which the study by Reisman *et al.* (1975) differed from ours. They did not test for wasp-sensitive subjects; they discarded serums obtained more than 3 years after a systemic reaction; and they used venom, not venom sacs, in all their RAST determinations.

With clear historical information of a systemic reaction following a Hymenoptera sting, it was surprising to find a normal IgE antibody level in nearly half of the patients tested. From our data, there is no ready explanation for this finding, but several possibilities exist. The waning of anaphylactic sensitivity with time has been previously found in penicillin-sensitive patients (Levine, 1966). In addition, Reisman *et al.* (1976) have noted a similar decline in IgE venom antibodies as the interval between stings is increased. While normal levels of IgE antibody were found more frequently in patients with reactions occurring over 10 years ago (Table 3), the number of observations involved is too small to be meaningful. It also does not explain the normal IgE levels found in those individuals tested soon after their systemic reaction.

On the other hand, we found that where the insect could be clearly identified, as in the case of honey bee sting reactions, the correlation between history, venom skin

test and RAST was good (Table 5). This may not indicate a greater degree of clinical sensitivity in the honey bee-sensitive patients, but may be a function of the larger available quantity of honey bee venom used in preparation of the honey bee venom RAST reagent. Moreover, an unknown proportion of the venom sac extracts represented non-allergic protein derived from the sac wall rather than the venom within the sac, which served to reduce further the allergen density on the solid phase RAST reagent.

It is also possible that there exists subspecies antigenic variation within the various Hymenoptera species. Preparation of the RAST reagents may therefore have involved one subspecies antigen and the patient had a reaction to a different antigenically distinct venom. While over seventeen subspecies of *Polistes* have been reported, recent studies by Findlay *et al.* (1977) with venom from three *Polistes* subspecies indicated almost total allergenic similarity. Whether this exists with the other subspecies or Hymenoptera species is unknown.

Absence of elevated venom IgE antibodies in patients reporting insect sting allergy is consistent with recent findings by investigators at Johns Hopkins (Valentine *et al.*, 1977). From studies of 182 patients who considered themselves highly allergic to insect stings, only fifty-nine had both an unequivocal history of anaphylaxis and a positive immediate skin test response to at least one of five venoms employed. Data on the RAST in these individuals was not provided, however.

Our data indicate that a significant number of the patients studied had no elevation in serum IgE antibodies to Hymenoptera venoms, in spite of a clear-cut history of anaphylaxis. Do these represent false negative RAST results? In contrast to the availability of the RAST for all physicians, other diagnostic tests, such as the leucocyte histamine release procedure, are only research tools at the present time. Deliberate sting challenges are not practical from either the ethical or medicolegal standpoint. Given this dilemma, the conservative approach is to consider all patients with histories of systemic reactions in the past to be potential candidates for anaphylaxis in the future, and to provide these patients with emergency kits with epinephrine. However, without other *in vitro* tests or venom skin tests to corroborate such a clinical history, this approach may simply increase needless concern and contribute to overtreatment or useless treatment with whole insect body immunotherapy (Lichtenstein, Valentine & Sobotka, 1974; Busse *et al.*, 1975). Our findings indicate a compelling need for potent Hymenoptera venom skin test reagents to more completely define and diagnose the patient with suspected Hymenoptera anaphylaxis.

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