

The evaluation of the common diagnostic methods of hypersensitivity for bee and yellow jacket venom by means of an in-hospital insect sting

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Between 1979 and 1983 230 patients visited our clinic in connection with allergic reactions after insect stings. One hundred six patients were subjected to a diagnostic provocation test with a live insect; 86 of these patients had a history of systemic reactions and a positive skin test and RAST with insect venom. Thirty-one of these patients, including one patient with a negative RAST and another with a negative skin test, demonstrated a generalized reaction and were subjected to immunotherapy with pure insect venom. Comparison of the diagnostic data from 31 patients with reactions with those of the 57 nonreacting patients from the 86 patients aforementioned reveals that at this time only a provocation test with a live insect can provide the evidence of an allergy to insect venom leading to such a severe generalized reaction that admission to probably lifelong immunotherapy is justified. The measurement of the venom-specific IgG, the ratio of IgG/IgE, and (for bee patients) the serum antibody titer against the bee venom components phospholipase A and hyaluronidase did not improve the diagnosis of a current hypersensitivity against insect venom. (J ALLERGY CLIN IMMUNOL 75:556-62, 1985.)

In recent years the interest in hypersensitivity reactions caused by stings of Hymenoptera (mainly bees and wasps living in colonies) has increased noticeably. It has been estimated by Frazier¹ and other authors^{2,3} that eight out of 1000 people exhibit allergic reactions after being stung, and half of these cases are very serious. Charpin⁴ even reported a possible incidence of 4%.

Data about the number of cases that have resulted in death are known for a number of countries: United States, ± 40 per year^{5,6}; Switzerland, 3.6 per year⁷; France, 12.5 per year⁸; Great Britain, 4.6 per year⁹; Denmark, 1.4 per year¹⁰; and the Netherlands ± 2 per year.¹¹ Although the number of cases per year that result in death is quite small, it has become evident to us that many people, nonetheless, remain very anxious about the possible consequences of a future sting after experiencing a more or less serious reaction.

Abbreviations used

PLA: Phospholipase A

Hy: Hyaluronidase

Since 1978 a number of authors¹²⁻¹⁴ have reported that immunotherapy with bee venom for patients hypersensitive to Hymenoptera venom almost always has provided complete protection. The problem is, however, which selection criteria should be used to determine which patients should come under consideration for this adequate but probably lifelong therapy. Hunt et al.¹² had already reported in 1978 that approximately 40% of the patients treated with a placebo preparation or a whole insect body extract did not exhibit further systemic reactions after a provocation test with a live insect.

We report in this article our experiences with diagnostic tests to establish allergy to insect venom. History, skin tests, and venom-specific IgE levels are compared with the outcome of diagnostic insect provocation tests performed at the same time. Since many authors^{15,16} credit the serum venom-specific IgG with

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TABLE I. The composition of the patient groups I and II

	Provocation positive group I (n = 31)		Provocation negative group II (n = 57)	
	bee	wasp	bee	wasp
No.	19	12	19	38
Age (yr) ($\bar{X} \pm 1$ SD)*	42 $\pm$ 15	46 $\pm$ 11	37 $\pm$ 10	36 $\pm$ 12
Range (yr)	11 to 68	24 to 65	18 to 59	14 to 61
% Male	58	17	58	47
No. beekeepers or direct relation†	15	0	15	0

*No significant difference in age ($p > 0.05$).

†Family member living in same house.

a protective function against anaphylactic reactions, we constantly measured this parameter of potential diagnostic value.

MATERIAL AND METHODS

Patients

Between May 1979 and August 1983 230 people visited our clinic with a history of allergic systemic reactions after insect stings.

According to the guidelines of the American Academy of Allergy Committee on Insects,¹⁷ 86 of these patients were eligible for immunotherapy on the basis of a positive history, skin tests with bee and/or yellow jacket venom, and increased serum venom-specific IgE levels. These patients were subjected to a diagnostic provocation test with a live insect. Another 20 patients (all of them adults), who did not meet all the criteria mentioned above, were also challenged for various reasons such as anxiety for future stings, great occupational risks, etc. In every case the patients permission was obtained in accordance with the guidelines established in the Declaration of Helsinki (1975).

History

The generalized reactions reported in the patients' history were divided into gradations of seriousness ranging from I to IV, according to the method of Mueller,¹⁸ in which I represents a light and IV a severe generalized reaction (shock). The data in the patients' history usually could be supported by heteroanamnesic data originating from doctors of other clinics involved in previous medical treatments of the patients.

Skin tests with insect venom

The skin tests were performed by intracutaneous injection with 0.05 ml of venom solution of increasing strength, beginning with 0.01 μ g/0.5 ml. The bee venom, 5 mg, (quality, Sigma Chemical Co., St. Louis, Mo.) originated from Diephuis (Groningen, The Netherlands) and was dissolved in Coca fluid and diluted with this solvent to concentrations of 0.01, 0.1, 1.0, and 10.0 μ g/0.5 ml, respectively.

The yellow jacket venom was supplied by Pharmacia Diagnostics (Uppsala, Sweden) and was dissolved in the accompanying diluent (normal serum albumin), also in con-

centrations of 0.01, 0.1, 1.0, and 10.0 μ g/0.5 ml. To establish the concentration at which toxic skin reactions appeared, 30 healthy volunteers varying in age from 20 to 35 yr without a previous allergic history were subjected to skin tests with these venom solutions.

Volunteers demonstrating excessive skin reactions were excluded by skin tests performed with Coca fluid as well as histamine solutions in the concentrations of 0.1, 0.01, and 0.001 mg per milliliter.

Only two of the 30 volunteers developed an induration of ≥ 10 mm (measured after 15 min) for bee venom in the concentration of 1.0 μ g/0.5 ml.

Three of the volunteers responded with the development of an induration of ≥ 10 mm in diameter for yellow jacket venom in the concentration of 0.1 μ g/0.5 ml.

On the basis of these data, the outcome of the skin tests was evaluated as follows: bee venom (1) a score of 0 if the induration was < 10 mm in diameter at a concentration of 1.0 μ g/0.5 ml or (2) a score of 1, 2, or 3 if the induration was ≥ 10 mm in diameter at concentrations of 1.0, 0.1, and 0.01 μ g/0.5 ml, respectively; yellow jacket venom (1) a score of 0 if the induration was < 10 mm in diameter at a concentration of 0.1 μ g/0.5 ml or (2) a score of 1 or 2 if the induration was ≥ 10 mm in diameter at concentrations of 0.1 and 0.01 μ g/0.5 ml, respectively.

Venom-specific IgE and IgG determinations and the ratio IgG/IgE

Before the beginning of the diagnostic insect provocation test, the venom-specific IgE levels were determined twice according to the Phadebas IgE RAST method (Pharmacia Diagnostics). The IgE values mentioned were determined in blood samples collected immediately before the start of the provocation test.

The venom-specific IgG levels (Phadebas IgG RAST method) of all patients were determined in the same blood samples. However, these samples were always analyzed in one run because of the large between-run variation coefficient of $\pm 50\%$ (within run variation coefficient $\pm 15\%$).

A serum venom-specific IgE value ≥ 0.25 Phadebas RAST units per milliliter (mean $+ 2 \times$ SD of a control group) was considered positive for both bee and yellow

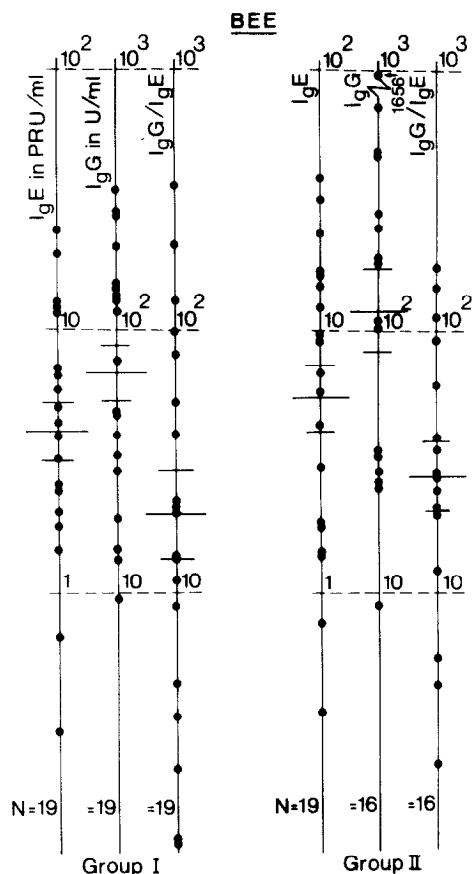


FIG. 1. Bee venom-specific IgE and IgG levels, the ratio of IgG/IgE, and geometric mean $\pm$ SEM for patient groups I (reactors) and II (nonreactors). No significant difference: $p > 0.05$.

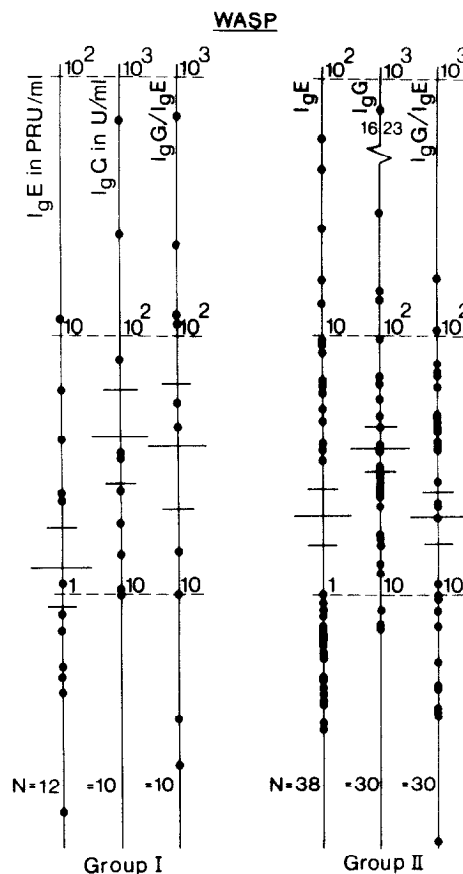


FIG. 2. Wasp venom-specific IgE and IgG levels, the ratio of IgG/IgE, and geometric mean $\pm$ SEM for patient groups I (reactors) and II (nonreactors). No significant difference: $p > 0.05$.

jacket venom. In the same way a value of 10 units per milliliter was maintained as the upper normal limit for the specific IgG level. The ratio of IgG/IgE was calculated from the determined IgG and IgE values.

The serum samples were always stored at -20°C . The stability of the IgG during the research period was sufficient at this temperature.

Determination of the antibody activity in the blood against the bee venom components PLA and Hy

The serum antibody titer against PLA and Hy for a number of patients hypersensitive to bee venom was determined by the Central Laboratory of the Blood Transfusion Service in Amsterdam* in two different manners: (1) PLA and Hy, respectively, were coupled to Sepharose. The respective suspensions were incubated with patient serum, washed, and then incubated with, respectively, ^{125}I -PLA and ^{125}I -Hy. The radioactivity of the solid phases was counted. (2) Protein A-Sepharose was subsequently incubated with patient

serum, ^{125}I -PLA and ^{125}I -Hy, respectively, and washed. The radioactivity of the solid phases was counted. The results for the sandwich and the protein A-Sepharose methods are expressed as a percentage of the total added radioactivity. The results are always obtained from blood samples taken immediately before the insect sting.

Other tests

All patients were subjected to a general physical examination while the following laboratory tests were done: total IgE serum level (Phadcbas), erythrocyte sedimentation rate, hemoglobin content, leukocyte differentiation, liver and kidney function tests, determination of immunoglobulin A, G, and M, the complement titer CH_{50} , and urine routine tests. In the group of patients who were examined, no results deviating from normal were found except for total IgE levels in some cases.

The provocation test with a live insect

Under intensive care conditions the patient was stung by a single bee or yellow jacket and, in the case of doubtful reactions, by several bees or yellow jackets. The bees all belonged to the species *Apis mellifera*. For the tests with

*Aalberse RC, et al: Centraal Laboratorium van de bloedtransfusiedienst. Amsterdam, The Netherlands.

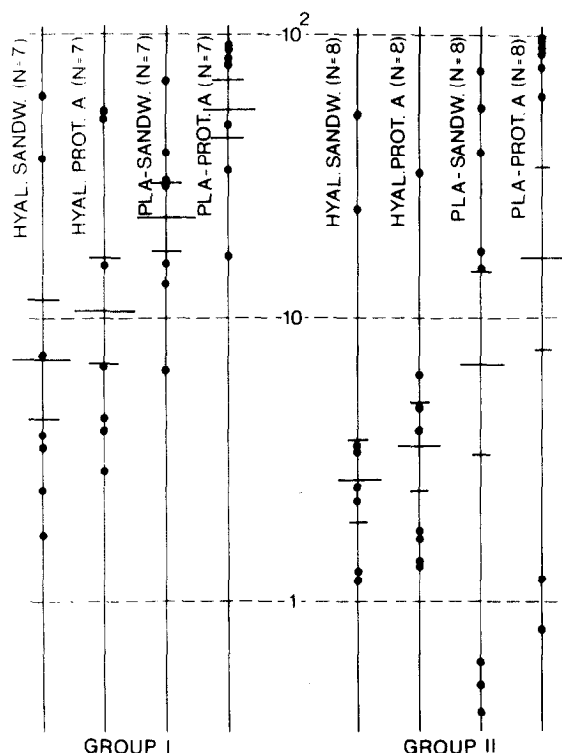


FIG. 3. The serum antibody activities (in percentage of the total added radioactivity) against the bee venom components PLA and Hy in a limited number of persons from the patient groups I (reactors) and II (nonreactors). No significant difference: $p > 0.05$.

the yellow jackets, the species *Vespula saxonica* and *V. vulgaris* were used in 1980, whereas in the following years, the species *V. vulgaris* and *V. germanica*¹⁹ were used. The use of different types of yellow jackets is acceptable because a large cross-reactivity exists between yellow jacket venom from different types.²⁰⁻²⁴

On the basis of the appearance or nonappearance of generalized reactions (type Mueller I to IV) during the provocation test, the patients were divided into two groups: group I, the reactors, who came into consideration for immunotherapy with venom, and group II, the nonreactors, who were not admitted to immunotherapy.

Statistical analysis

Groups I and II were divided into patients with bee and yellow jacket stings. The data obtained for both groups, such as age, serum venom-specific IgE and IgG levels, and ratio IgG/IgE were compared with the Mann-Whitney U test as well as the Student t test in order to establish statistically significant differences ($p < 0.05$). Use was made of the chi-square and the four-field test according to Fischer for comparison of the scores of the skin test and the severity of the history.

RESULTS

The characteristics of 106 patients who had diagnostic provocation tests are mentioned in this study.

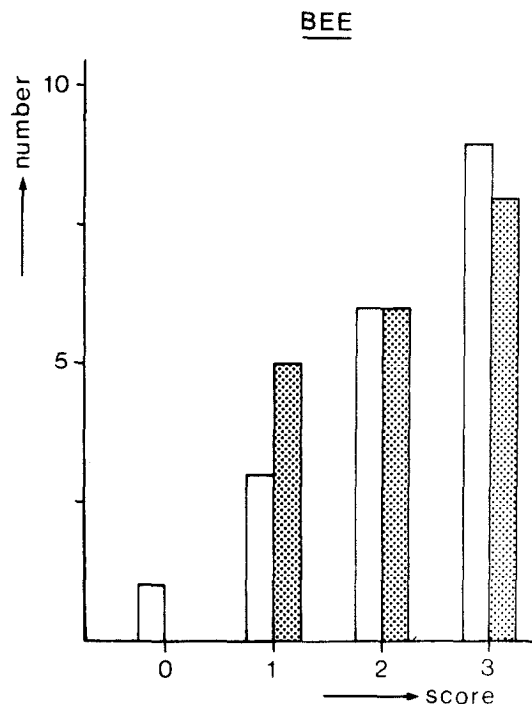


FIG. 4. Number of times a certain skin test score for bee venom occurred in bee-sting patient groups I (reactors) and II (nonreactors); group I ($\square$), $n = 19$; group II ($\blacksquare$), $n = 19$. No significant difference: $p > 0.05$.

Eighty-six patients did satisfy the strict requirements for the administration of immunotherapy established by the Committee on Insects¹⁷: a history of systemic reactions, a positive skin test with venom, and an increased serum venom-specific IgE. Within this group of patients, 29 demonstrated a generalized reaction to the sting challenge, whereas 57 patients did not.

Within the group of 20 patients who did not satisfy all three requirements aforementioned, two patients suffered from a systemic reaction after the sting challenge. One of these patients had a normal venom-specific IgE level, and the other patient had a negative skin test with sting venom. In total 31 patients demonstrated a generalized reaction (group I). These patients were subjected to immunotherapy with pure insect venom.

A comparison was made between group I and the 57 nonreacting patients, fully satisfying the requirements of the Committee (group II), in order to evaluate the importance of the parameters included in these requirements (Table I).

The values of the bee and yellow jacket (wasp)-specific IgE and IgG levels as well as the ratio of these levels for the groups I and II at the moment of provocation with a live insect are presented in Figs. 1 and 2.

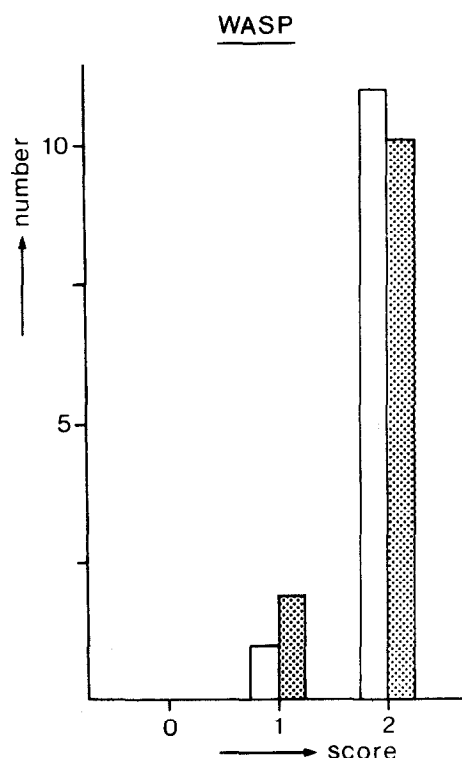


FIG. 5. Number of times a certain skin test score for wasp venom occurred in the wasp-sting patient groups I (reactors) and II (nonreactors); group I ($\square$), $n = 12$; group II ($\boxtimes$), $n = 38$. The numbers are always normalized to the lowest number of patients. No significant difference: $p > 0.05$.

The values of the serum antibody activities against the bee venom components Hy and PLA at the moment of the bee provocation test in a limited number of persons from patient groups I and II are presented in Fig. 3.

The number of times a certain skin test score for bee and yellow jacket (wasp) venom was found in groups I and II is presented in Figs. 4 and 5. The number of times that a certain degree of severity of a systemic reaction (according to the method of Mueller¹⁸) was found in the history of groups I and II is presented in Figs. 6 and 7.

DISCUSSION

Several authors estimate that patients, having experienced an earlier severe generalized reaction to an insect sting, have a 40% to 60% chance not to respond to a future sting.^{12, 22, 25, 26} This percentage for children was estimated by Schuberth et al.²⁰ to be 90%.

From the data in Table I, it can be observed that 57 of the 86 persons (66%) who were eligible for immunotherapy with insect venom according to the recommendations of the Committee on Insects¹⁷ did not react to a provocative insect sting.

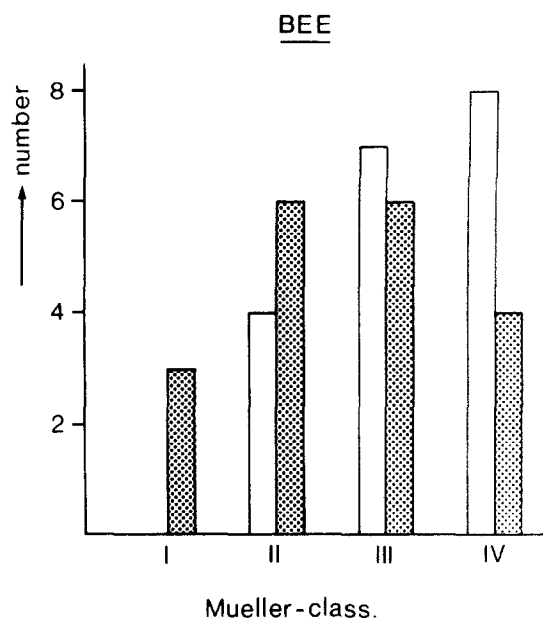


FIG. 6. Number of times a certain grade of severity of a systemic reaction was found in the history of bee-sting patient groups I (reactors) and II (nonreactors); group I ($\square$), $n = 19$; group II ($\boxtimes$), $n = 19$. No significant difference: $p > 0.05$.

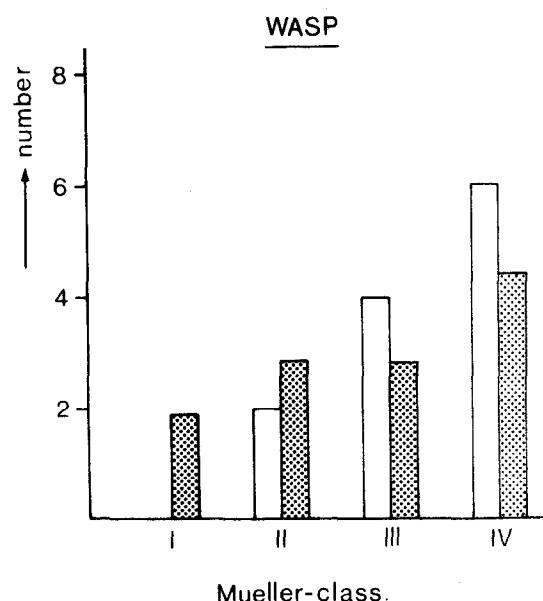


FIG. 7. The number of times a certain grade of severity of a systemic reaction was found in the history of wasp-sting patient groups I (reactors) and II (nonreactors); group I ($\square$), $n = 12$; group II ($\boxtimes$), $n = 38$. The numbers are always normalized to the lowest number of patients. No significant difference: $p > 0.05$.

Among the group of 31 patients who demonstrated a generalized reaction to the sting challenge, there was one yellow jacket-hypersensitive patient with a normal venom-specific IgE level and one bee-hypersensitive patient who had a negative skin test for bee

venom. These two patients reacted to the provocation with a severe systemic reaction (grade of severity IV).

It is clear from our data that, on the basis of the history, skin tests with venom, venom-specific IgE and IgG levels, the ratio IgG/IgE, and the level of the serum antibody activity against the bee venom components PLA and Hy, it is not possible to predict the occurrence of a patient's reaction to an insect sting. At this moment we have no explanation of this discrepancy between immunologic reactivity and clinical sensitivity. The instability of the kinin system or the severity of the stress situation at the moment of the sting may enhance the clinical consequences of an insect sting.²⁷

To determine if a sensitivity could develop among the group of nonreactors (group II) as a result of the sting challenge, the 57 patients belonging to this group were observed regularly by us as outpatients, and at every visit the IgE and IgG serum levels were assayed.

After 6 mo they were subjected once again to skin tests with insect venom. At least 10 of these patients were spontaneously stung outside the hospital by bees or yellow jackets without suffering serious symptoms. Our present data, collected in a follow-up period ranging from 9 mo to 3 yr, do not indicate that these patients became increasingly allergic to insect venom, which will be reported shortly.

Within the group of patients who generated a clear generalized reaction to a provocative sting, 39% responded to yellow jacket venom, whereas a 61% response to bee venom occurred. The number of bee-sensitive patients in the group of 86 eligible for immunotherapy were 80% beekeepers. Beekeeping is therefore a predisposing factor for the development of an immunologic reaction and/or clinical sensitivity to bee venom.

Finally, it can be concluded that at this time only a provocation test with a live insect can provide evidence for the existence of an allergy for insect venom with severe generalized reactions. In our view the decision to start with immunotherapy, made on the basis of the parameters formulated by the Committee on Insects, is often a wrong one. No other value can be ascribed to skin tests and the venom-specific IgE levels than that they are useful in establishing the identity of the insect that stung the patient in the past.

The determination of the venom-specific IgG or the ratio of IgG/IgE is of no value in establishing the degree in which an allergic reaction will appear after a future sting.

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