

Insect sting allergy with negative venom skin test responses

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Background: In our 1976 controlled venom immunotherapy trial, 33% of 182 patients with a history of systemic reactions to insect stings were excluded because of negative venom skin test responses. There have been reports of patients with negative skin test responses who have had severe reactions to subsequent stings.

Objective: Our aim is to increase awareness about the patient with a negative skin test response and insect sting allergy and to determine the frequency and significance of negative skin test responses in patients with a history of systemic reactions to insect stings.

Methods: We prospectively examined the prevalence of negative venom skin test responses in patients with a history of systemic reactions to stings. In patients who gave informed consent, we analyzed the outcome of retesting and sting challenge.

Results: Of 307 patients with positive histories screened for our sting challenge study, 208 (68%) had positive venom skin test responses (up to 1 µg/mL concentration), and 99 (32%) had negative venom skin test responses. In 36 (36%) of the 99 patients with negative skin test responses, the venom RAST result was a low positive (1-3 ng/mL), or repeat venom skin test responses were positive; another 7 (7%) patients had high venom-specific IgE antibody levels (4-243 ng/mL). Notably, 56 (57%) of 99 patients with positive histories and negative skin test responses had negative RAST results. In patients with positive skin test responses, sting challenges were performed in 141 of 196 patients, with 30 systemic reactions. Sting challenges were performed on 37 of 43 patients with negative skin test responses and positive venom-specific IgE and in 14 of 56 patients with negative skin test responses and negative RAST results. There were 11 patients with negative skin test responses who had systemic reactions to the challenge sting: 2 had negative RAST results, and 9 had positive RAST results at 1 ng/mL. The frequency of systemic reaction was 21% in patients with positive skin test responses and 22% in patients with negative skin test responses (24% in those with positive RAST results and 14% in those with negative RAST results).

Conclusions: Venom skin test responses can be negative in patients who will subsequently experience another systemic sting reaction. Venom skin test responses are negative in many patients with a history of systemic allergic reactions to insect stings and may be associated with positive serologic test responses for venom-specific IgE antibodies (sometimes

strongly positive results). Venom skin test responses should be repeated when negative, along with a serologic IgE antivenom test. Better diagnostic skin test reagents are urgently needed. (*J Allergy Clin Immunol* 2001;107:897-901.)

Key words: Skin test, insect venom, RAST, insect sting, Hymenoptera, anaphylaxis

Intradermal skin testing with Hymenoptera venoms is the recommended diagnostic method for confirmation of a positive insect sting allergy history before initiating venom immunotherapy. Although allergists expect that the venom skin test responses will be positive in the majority of patients with a clear history of insect sting anaphylaxis, some reports describe negative venom skin test responses in up to 30% of patients with a convincing history.¹⁻⁴ Some of these patients experienced a reaction decades earlier and may have lost their sensitivity, but others report a recent severe reaction. The venom RAST is considered less sensitive than skin tests, and therefore allergists often assume that performing the RAST is unnecessary when skin test responses are negative. It has also been commonly assumed that negative venom skin test responses indicate that there is no risk of systemic reaction to a sting. However, there have been occasional reports over the past 20 years of patients with negative venom skin test responses who experienced severe reactions to subsequent stings.²⁻⁸ We have reported that patients who discontinue venom immunotherapy can subsequently have systemic reactions to stings, even after they have negative venom skin test responses.^{9,10} We therefore have focused on untreated patients with a history of systemic reactions to stings who have negative venom skin test responses. The guidelines, position statements, and practice parameters relating to insect sting allergy give very little guidance for managing these patients.¹¹⁻¹³ Our aim was to determine the frequency and significance of negative skin test responses in patients recruited for our current sting challenge trial. We also aimed to increase awareness of this problem so that patients can be tested and advised in an appropriate fashion.

METHODS

Candidates for our sting challenge trial who responded to approved advertisements were interviewed. Subjects with a history consistent with IgE-mediated systemic allergic reactions were evaluated by means of venom skin testing and serologic IgE antivenom assays. Sting challenge was initially limited to subjects with a positive venom skin test response or RAST result. Patients with no detectable venom-specific IgE antibodies determined by either method were offered repeat skin tests and blood tests. A diagnostic sting challenge was offered to those with recent convincing histories.

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Intradermal venom skin tests were performed with 5 Hymenoptera venoms (ALK-Abello Laboratories, Wallingford, Conn) in concentrations from 0.001 $\mu\text{g/mL}$ to 1.0 $\mu\text{g/mL}$. Venom-specific IgE antibody was detected in serum by means of RAST, as previously described,¹⁴ with an analytic sensitivity of 1 ng/mL. Sting challenge was performed, as previously described,¹⁵ with the insect implicated by the history (and/or skin test-RAST) under medical supervision in a National Institute of Health-supported General Clinical Research Center, after obtaining informed consent. A systemic reaction to the sting challenge was defined as either mild (scattered hives, drop in peak expiratory flow of 15%-20%, and mild hypotension with systolic blood pressure of greater than 90 mm Hg), moderate (severe generalized urticaria and angioedema, drop in peak expiratory flow of 20%-30%, and systolic blood pressure of 70-90 mm Hg), or severe (unconsciousness or systolic blood pressure of less than 70 mm Hg, drop in peak expiratory flow of >30%, or severe respiratory distress).

Reaction rates to challenge stings were compared with rates of positive skin test responses or RAST results (or sampled) on the date of the sting challenge by using the Fisher exact test.

RESULTS

During 4 years of recruiting for the study, 307 subjects had a history of a systemic reaction to a sting, which was sufficiently convincing to justify a screening visit and diagnostic testing (Table I). Intradermal venom skin tests up to 1 $\mu\text{g/mL}$ elicited negative responses in 99 (32%) of the subjects, of whom 56 (57%) had a negative RAST result and 43 (43%) had a positive RAST result (36 with a low-level RAST result from 1-3 ng/mL and 7 with high venom-specific IgE levels from 4-243 ng/mL).

Sting challenge was performed on a total of 51 of the 99 patients with negative skin test responses. We stung 37 of 43 patients with negative venom skin test responses who had a positive RAST result, and 14 of 56 patients with both negative venom RAST results and skin test responses were selected because of a more convincing or recent reaction history (Table II). Systemic reactions occurred in 11 of these 51 patients with negative skin test responses: 9 had venom-specific IgE antibody levels of 1 ng/mL, and 2 were IgE negative. All systemic reactions presented objective signs accompanying the symptoms, including reduced blood pressure with dizziness, reduced peak expiratory flow rate with dyspnea or tightness in the throat or chest, urticarial lesions or angioedema, or flushing with generalized pruritus. The reactions to challenge sting were classified as mild in 7 patients, moderate in 4 patients, and severe in none. Our studies have focused on yellow jacket allergy, and 49 of the 51 patients with negative skin test responses stung had yellow jacket stings. Two patients had a *Polistes* species wasp sting on the basis of history and RAST. Thirteen of the patients stung with yellow jackets also had stings on a different day with other species on the basis of uncertain history or positive RAST result. All systemic reactions occurred with yellow jacket stings.

The 22% reaction rate in the 51 patients with negative skin test responses was not significantly different from the 21% frequency of systemic reactions to sting challenges in 141 patients with positive skin test responses. The reaction

rate was higher in patients with negative skin test responses with positive RAST results (24%) than in those with negative RAST results (14%), although the difference was not statistically significant with the small number of sting challenges in some subgroups.

We also evaluated the past history as a possible factor in the reaction rate. There was no significant difference in the severity of the history between the patients with positive skin test responses and those with negative skin test responses. In both groups the history of systemic reaction was mild in approximately 25%, moderate in 55%, and severe in 20% of the patients. In the patients with negative skin test responses, 6 systemic reactions occurred in those with a past history of mild systemic reaction, 4 reactions occurred in those with a previous moderate reaction, and 1 reaction occurred in a patient who previously had a severe reaction. There was no significant difference between the groups with positive and negative skin test responses in the time elapsed since the last systemic reaction.

DISCUSSION

The state of the art when Hymenoptera venoms were approved by the Food and Drug Administration in 1979 stated that "patients with a history of a systemic reaction but negative venom skin tests ... most critically need further study ... (and) require careful follow-up, but no immunotherapy."¹⁶ The existence of patients with negative venom skin test responses who had systemic reactions to insect stings was documented early,⁴⁻⁶ with a "plea to the allergist" not to ignore an anaphylactic history because of a negative skin test response.⁵ However, in other reports negative skin test responses were often construed to mean that sensitivity was lost with time.³ Published practice guidelines^{11,12} and parameters¹³ state that patients with negative skin test responses are not candidates for immunotherapy, but they provide no guidance for managing these patients. We have reported that after discontinuing venom immunotherapy, systemic reactions occurred at the same frequency in patients with negative and positive skin test responses.¹⁰ Our results now indicate that also in untreated patients, there is a similar reaction rate in patients with negative and positive skin test responses. The fact that 32% of our patients with a positive history had negative venom skin test responses is quite consistent with other reports,¹⁻⁴ but it also reflects some self-selection factors in this patient population.¹⁷ Our data indicate that negative skin test responses in this population were not necessarily caused by a loss of sensitivity with time or a milder degree of reactions to stings in those patients.

Skin tests with Hymenoptera venoms were described 25 years ago as having high clinical specificity compared with whole-body extracts.^{3,18,19} However, venom-specific IgE antibodies in the serum (RAST) were negative in over 20% of patients with positive skin test responses, and conversely, the RAST result was positive (often strongly positive) in 11% to 16% of patients with nega-

tive skin test responses.^{14,20} These correlations are quite similar to those reported for other allergens. In this study patients with positive skin test responses had negative RAST results in 20% of patients. Among the patients with negative skin test responses in this study, 43% had positive venom-specific RAST results, some of which were quite elevated. The frequency of the positive RAST result in patients with negative skin test responses with a positive history is twice as high as in previous reports. This may reflect the ability of our RAST assay to detect levels of venom-specific IgE as low as 1 ng/mL. Because of differences in methodology, it is not possible to extrapolate our results to other venom antibody assays performed by other laboratories. The discordance between our skin test and RAST results could also be due to differences in the materials used. Our RAST assay requires the use of precommercial venom preparations, which do not contain additional components that interfere in the assay. The commercial and precommercial venom preparations are from the same source (Vespa laboratories), and we have reported that comparisons with histamine release assays showed no difference in the potency of these materials.¹⁷

The difference in reaction rates between patients with negative and positive skin test responses may be underestimated in this study because of selection factors in the patients with negative skin test responses who were stung and in the study population as a whole. Of the patients with both negative skin test responses and RAST results, relatively few had a challenge sting. Those stung were generally self-selected or selected by the physician because their history was of particular concern. It is therefore possible that if a challenge sting had been performed on all 99 of the patients with positive histories and negative venom skin test responses, the overall frequency of systemic reactions could have been as low as 11% overall and as low as 4% in those who also had a negative RAST result. Although the reaction rate does appear to be lower in the patients with negative skin test responses and negative RAST results than in those with positive RAST results, this difference is not statistically significant because of the small number of patients and reactions in the group with negative RAST results. In the patients with positive skin test responses on the other hand, our 24% reaction rate is less than the 40% to 60% of reaction rates previously reported.^{1,21,22} This is in part because this study population as a group had less severe insect allergy than in our previous studies (we also excluded patients with the most severe near-fatal reactions),^{1,17} and the severity of the clinical history is an important predictor of the frequency and severity of a reaction to a challenge sting.^{8,23-25} However, all patients had a history convincing enough that clinicians would have to consider venom immunotherapy, and in many cases the reaction was treated in an emergency department. Whether the risk of a systemic reaction to a sting in patients with negative skin test responses is 11% or 22%, our results clearly indicate to physicians that negative test results do not exclude the possibility of a reaction.

TABLE I. Venom skin test-IgE in patients with positive histories

Positive skin test response	208 (68%)
Negative skin test response	99 (32%)
Negative skin test response/negative RAST result	56 (57%)
Negative skin test response/positive RAST result	43 (43%)
RAST, 1-3 ng/mL	36
RAST, 7-243 ng/mL	7

There were a total of 307 patients with positive histories who were screened.

TABLE II. Sting challenge reaction in patients with positive histories

	Stung	Systemic
Positive skin test responses (n = 208)	141	30 (21%)
Negative skin test responses (n = 99)	51	11 (22%)
Negative RAST result (n = 56)	14	2 (14%)
Positive RAST result (n = 43)	37	9 (24%)

Systemic reactions in patients with negative venom skin test responses may reflect limited diagnostic sensitivity of the skin test for IgE-mediated sensitivity or could indicate nonallergic reactions, such as anxiety-panic, conditioned reflex reactions, or toxic reactions. Although patients with mastocytosis may have systemic reactions in the absence of venom-specific IgE antibodies, most do have detectable positive RAST results.^{26,27} However, only one of our patients with negative skin test responses who reacted to challenge sting had elevated baseline serum tryptase levels. The serum tryptase level was increased at the time of the reaction in most, but not all, of the patients with positive skin test responses having systemic reactions. In the patients with negative skin test responses, only 1 of 11 reactors had an elevated serum tryptase level during the reaction: he had the most severe history of this group of patients, but his challenge reaction was mild. The lack of detectable tryptase in most of these reactors is consistent with their relatively milder histories and reactions compared with skin test positive reactors.

Our results indicate that current diagnostic skin test reagents lack the necessary clinical sensitivity. The ability to detect low levels of venom-specific IgE antibodies in the skin with commercially available venom preparations is restricted by the limited range of concentrations that can be used because of the irritative effects at concentrations higher than 1 µg/mL. Unfortunately, low levels of venom-specific IgE antibodies (approximately 1 ng/mL) can be associated with severe anaphylaxis. However, venom skin test responses are still relevant and useful because they are positive in at least 65% of adults with positive histories and in only 17% of adults with negative histories.²⁸ The relative frequency (but not the severity) of reaction to a sting is related to the level of skin test or venom IgE sensitivity. In adults with no history of allergic sting reactions, a systemic reaction to subsequent stings occurred in 0 of 120 patients with negative venom skin test responses, whereas it occurred in

11 (17%) of 65 of those with a positive skin test response.²⁸ We estimate in this report that patients with positive histories have a 5% to 10% reaction rate if both venom skin test responses and RAST results are negative and up to a 20% reaction rate with negative venom skin test responses and a positive RAST result. When both the history and the venom skin test responses are positive, patients have a 20% to 70% reaction rate. This range correlates with the severity of the previous reaction and, to a lesser extent, with the level of skin test sensitivity.²³ The patient with a positive history and a negative skin test response therefore represents the lower end of the spectrum of venom sensitivity.

The patients with negative skin test responses in our studies reveal several clinical patterns. A small but discrete subset of patients have vascular anaphylaxis, often with abrupt onset of severe hypotension but without cutaneous or respiratory symptoms. They can have negative skin test responses and barely detectable venom IgE antibodies (approximately 1 ng/mL). Other patients have negative skin test responses but a positive RAST result, a weakly positive skin test response, or both when the tests are repeated 3 to 6 months later. This does not reflect a refractory period (as in patients who have had recent anaphylaxis) but rather a low level of sensitivity with skin test results that seem to fluctuate around the threshold of detection. Skin tests sometimes fail to detect substantial levels of venom-specific IgE, and they can vary over time in the same patient. We previously made similar observations in studies of the natural history of venom sensitization in untreated and asymptomatic individuals,^{20,28} as well as during studies of patients who have discontinued venom immunotherapy.^{9,10,15} The test results described here were always on the day of the sting challenge. However, there were a few cases in whom the pre-sting screening skin test response had been negative but the test response on the day of challenge was positive (without intervening stings). Such patients were counted as having positive skin test responses (on the day of the sting). In such cases the RAST results had been positive initially and remained positive. We believe this represents biologic variability in the patient rather than in the skin test material. The venom source for all skin tests performed in our studies is always ALK Laboratories, although the lot numbers may differ over time. We have reported our comparison of different lots of venom from 3 different years over a 15-year period and found no difference in activity by means of histamine release or RAST inhibition assays.¹⁷ ALK has also provided in-house data on the standardization of their venoms to demonstrate the consistency of the allergenic activity from batch to batch.

Our results and experience suggest that current skin test reagents for yellow jacket allergy have limited diagnostic sensitivity. Venom skin test responses are often negative in patients with a history of systemic allergic reactions to insect stings, even in some patients who will have a systemic reaction to a subsequent sting. A patient with insect allergy and negative venom skin test responses should be evaluated by means of RAST results and

repeat skin test responses after 3 to 6 months. Even when all test responses are negative, the patient should be advised that it is not possible to fully exclude the risk of a systemic reaction to a future sting and that appropriate precautions should be taken for avoidance and treatment of reactions. Improved *in vivo* diagnostic reagents with greater diagnostic sensitivity and reduced nonspecific irritation are needed.

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