



Immunologic Reactivity on One Year Follow-Up of Subjects without Allergy to *Hymenoptera* Stings

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Hymenoptera stings are common and sometimes deadly, with approximately 40 deaths per year being attributed to *Hymenoptera* stings in Japan.¹ In particular, *Hymenoptera* allergy in forestry workers has recently become a serious medical problem, and an accurate diagnosis of *Hymenoptera* allergy is crucial. The presence of venom-specific IgE, one of the diagnostic parameters for *Hymenoptera* allergy,^{2,3} is found in 12-16% of the general population.⁴⁻⁷ In addition, venom-specific IgE is frequently included as a criterion for performing or stopping venom immunotherapy, and used as parameter of immunologic reactivity during follow-up.⁸⁻¹⁰

There are many studies about the natural history of *Hymenoptera* stings, but almost all studies were performed on allergic subjects with systemic reactions to *Hymenoptera* stings; few studies on nonallergic subjects are available.^{7,11,12} Individuals with systemic reactions to

SUMMARY We studied *Hymenoptera* stings in 72 pest-control operators without any previous systemic reactions to *Hymenoptera* stings, and investigated their venom-specific IgE levels in serial specimens collected over one year. At the initial evaluation, venom-specific IgE was present in 25 (34.7%) of 72 pest-control operators, and venom-specific IgE titer significantly decreased as the time interval from the last sting increased ($p < 0.001$). In most cases, venom-specific IgE disappeared less than 3 years after the last sting. On the other hand, the ratio of subjects with positive CAP for venom-specific IgE was significantly increased with an elevation of total serum IgE level ($p < 0.001$). After the one year follow-up, venom-specific IgE titer in the 25 subjects with positive CAP decreased significantly ($p = 0.026$). Total serum IgE level modified the decline significantly ($p = 0.011$), but the time interval from the last sting did not. In elevated total IgE level (>250 IU/ml), the decline of venom-specific IgE tended to be slow.

Hymenoptera stings usually have tolerated prior stings with no difficulty,¹⁰ suggesting that nonallergic subjects have the potential for systemic reactions with further stings. To evaluate this potential risk, it is important to study the natural course of venom-specific IgE in subjects without allergic reaction to stings. Thus in the present study, we followed the decrease in venom-specific IgE titer in serial specimens from nonallergic subjects for one year.

MATERIALS AND METHODS

Subjects included 72 Japanese pest-control operators (all male) with a previous history of *Hymenoptera* stings and a mean age of 36.3 years (range: 21-57). All subjects were free from allergic reactions in their histories, as de-

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defined by completely tolerated or mild local reactions without any systemic reaction. Initial evaluation was performed during a routine medical examination between 1994 and 1995, and reevaluation was performed one year after the initial evaluation. During the one year follow-up, none of the subjects received venom immunotherapy or had a second sting.

At the initial evaluation, these 72 subjects were interviewed using a questionnaire, and blood samples were taken. The questionnaire included the following items: 1) the number of past *Hymenoptera* stings; 2) the interval since the last sting; 3) identification of the offending insects; 4) clinical symptoms; 5) individual atopic history. Items 3) and 4) related to the last sting-incident. Regarding identification of the offending insect, we asked the subjects to select from 3 subfamilies of *Hymenoptera*: *Apiinae* (honeybee), *Polistinae* (wasp) and *Vespinae* (yellow jacket and hornet). In classification of the clinical symptoms, local reaction was defined as localized pain, swelling and erythema at the sting site. Individual atopic history was defined to include atopic dermatitis, urticaria, asthma, or allergic rhinitis. Follow-up questionnaire was concerned with a history of further stings during the past one year, and a blood sample was taken again. All subjects provided informed consent before taking part in the study.

Sera were obtained from the blood samples. The total serum IgE and venom-specific IgE to a yellow jacket, a wasp, and a honeybee were measured by the CAP system using a commercial kit (CAP

RAST FETIA Kit, Pharmacia Diagnostics AB, Uppsala, Sweden). The CAP results of venom-specific IgE were expressed in UA/ml (UA is short for "unit allergen", which are equal to Phadebas RAST Units [PRU]). The CAP results defined 0.70 UA/ml or higher as positive, less than 0.35 UA/ml as negative, and between 0.35 and 0.69 UA/ml as borderline. Of the three kinds of venom-specific IgE, we used the one to the most recent sting, and when the offending insect could not be identified, the highest of the three was used.

Methods of statistical analyses used are described in the footnote of each table or each figure legend. A *p* value less than 0.05 was considered significant for each statistical test.

RESULTS

Table 1 summarizes the overall features of *Hymenoptera* sting in the 72 subjects at the initial evaluation. The number of past stings ranged from 1 to 10 with a mean of 2.8 times. The time interval from the last sting ranged from 4 months to 35 years with a mean of 10 years. Approximately 30% of subjects had been stung more than 15 years ago and 12.5% within one year. Twenty-seven subjects (37.6%) were stung by the *Polistinae* subfamily (wasp), and 15 subjects (20.8%) were unable to identify the offending insects. Total serum IgE was widely distributed from 5 to 8,900, with a geometric mean of 128 IU/ml. Atopic history was observed in 13 subjects (18.1%), and *Hymenoptera* venom-specific IgE was positive in 25 subjects (34.7%).

Table 1. Overall features of *Hymenoptera* stings in 72 subjects

Age (years)	36.3 (21-57) ^a
Number of past stings	2.8 (1-10) ^a
Time interval from the last sting (years)	10.0 (0.3-35.0) ^a
Total IgE level (IU/ml)	128.0 (5-8,900) ^b
Kind of offending insect ^c	
<i>Apiinae</i> (honeybee)	15 (20.8%)
<i>Vespinae</i> (yellow jacket and hornet)	15 (20.8%)
<i>Polistinae</i> (wasp)	27 (37.8%)
Unknown	15 (20.8%)
Atopic history	
Positive	13 (18.1%)
Negative	59 (81.9%)
Venom-specific IgE	
Positive (>0.70 UA/ml)	25 (34.7%)
Borderline (0.35-0.69 UA/ml)	3 (4.2%)
Negative (<0.35 UA/ml)	44 (61.1%)

^a Values are arithmetic means with ranges in the parentheses.

^b Value is geometric mean with range in the parenthesis.

^c Responsible for the most recent sting.

Table 2. Association between CAP results and several factors in 72 subjects at the initial evaluation

	Venom-specific IgE (UA/ml)		p value
	≥ 0.70 (N = 25)	<0.70 (N=47)	
Age (year) ^{a,d}	39.2 (21-57)	34.8 (21-57)	ns
Number of past stings ^{a,d}	3.6 (1-10)	2.3 (1-10)	ns
Time interval ^{a,d}	5.2 (0.3-20.0)	12.5 (0.3-35.0)	<0.001
Total IgE level (IU/ml) ^{b,d}	417 (51-8,900)	69 (5-1,000)	<0.001
Kind of offending insect ^{c,e}			
<i>Apinae</i> (honeybee)	4	11	ns
<i>Vespinae</i> (yellow jacket and hornet)	5	10	
<i>Polistinae</i> (wasp)	12	15	
Unknown	4	11	
Atopic history ^e			
Positive	7	6	ns
Negative	18	41	

^a Values are arithmetic means with ranges in the parentheses
^b Values are geometric means with ranges in the parentheses
^c Responsible for the most recent sting
^d by Mann-Whitney U-test; "ns" indicates "not significant" (p>0.05)
^e by Chi-square test

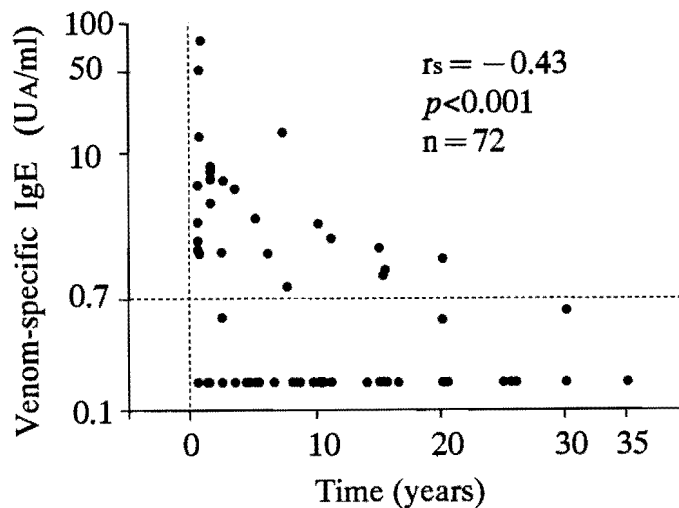
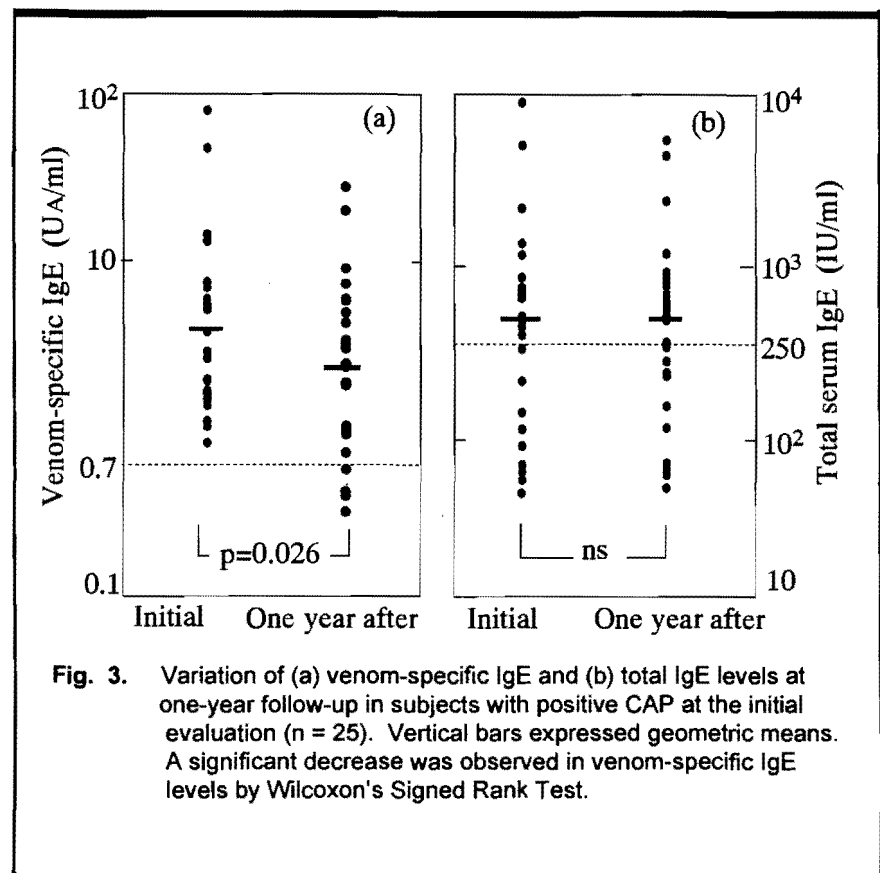
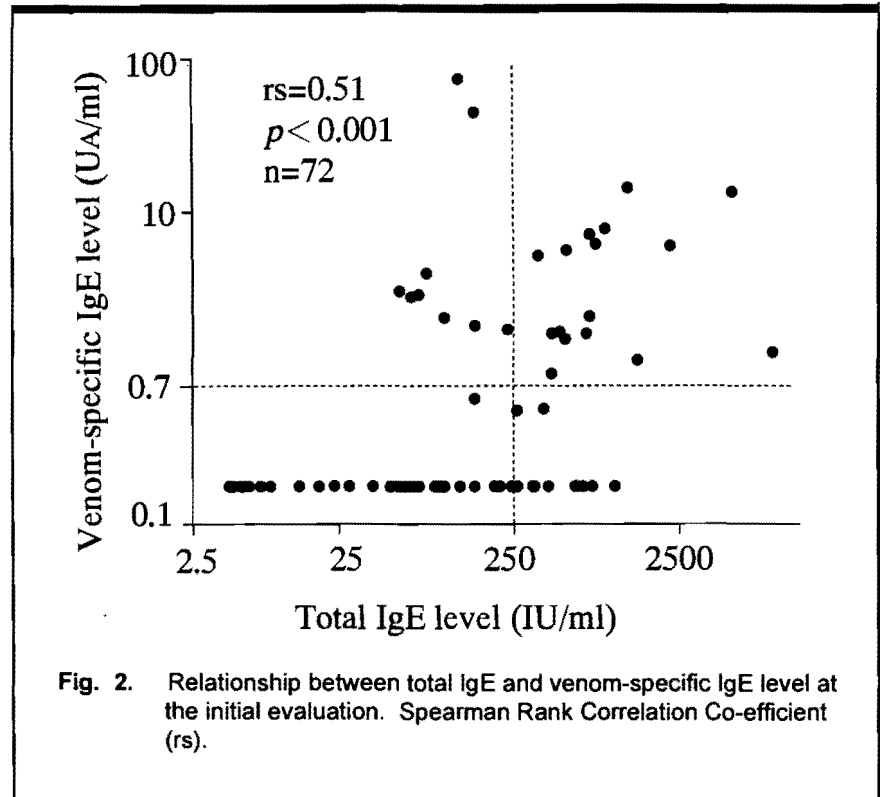


Fig. 1. The relationship between venom-specific IgE titer and time interval since the last sting at the initial evaluation. Spearman Rank Correlation Co-efficient (rs).

The association between these factors and CAP results is summarized in Table 2. Age and type of insect did not show any effect on the CAP results. Two factors, time interval since the last sting and total serum IgE level, were significantly associated with positive CAP results (both $p < 0.001$, by Mann-Whitney U test). In subject with positive CAP (venom-specific IgE titer ≥ 0.70 UA/ml), the mean of the time interval from the last sting was 5.2 years, which was shorter than that in those with negative CAP (venom-specific IgE titer < 0.70 UA/ml). Total serum IgE level was significantly higher in those with positive CAP, compared to those with negative CAP; a geometric mean was 417 IU/ml and 69 IU/ml, respectively. Number of past stings and atopic history had little effect on the CAP results; the subjects with positive CAP tended to have a higher number of past stings and a greater percentage had an atopic history. However, these influences were not significant ($p > 0.05$) by Mann-Whitney U test in the present study.

Fig. 1 shows the relationship between venom-specific IgE titer and the time interval since the last sting in 72 subjects at the initial evaluation. Venom-specific IgE titer decreased significantly with the passage of time. Spearman rank correlation co-efficient was calculated at -0.43 ; the relationship was significant ($p < 0.001$). The ratio of subjects with positive CAP varied at 3 years as the borderline; the positive rates were 70.0% (14/20) and 21.2% (11/52) in subjects stung less than 3 years earlier and in those stung 3 and more years earlier, respectively. Venom-speci-



fic IgE was even present in 6 subjects stung more than 10 years ago. Fig. 2 shows the relationship between venom-specific IgE titer and total serum IgE levels at the initial evaluation. The ratio of subjects with positive CAP was significantly increased with an elevation of total serum IgE. Spearman rank correlation co-efficient was calculated at 0.51; the relationship was significant ($p < 0.001$). However, the relationship was not clear among 25 subjects with positive CAP because several subjects who had been stung less than 3 years earlier showed a high value of venom-specific IgE titer irrespective of total serum IgE level.

Because CAP results remained negative at the follow-up evaluation in all 47 subjects with negative CAP at the initial evaluation, description of the follow-up evaluation is limited to the 25 subjects with positive CAP. Fig. 3 shows the changes in venom-specific IgE titer and total serum IgE level for one year in 25 subjects with positive CAP at initial evaluation. Venom-specific IgE titer decreased in 18 subjects during one year, and the level became insignificant in 4. The geometric mean decreased from 4.01 to 2.49 UA/ml for one year and this decrease was significant ($p = 0.026$) by Wilcoxon's signed rank test in spite of the presence of 7 subjects whose venom-specific IgE level was elevated. However, total serum IgE levels did not change over one year; total IgE levels decreased in 14 subjects and increased in 11 subjects and the geometric mean values were 417 and 391 IU/ml at the initial and follow-up evaluation, respectively.

Effect of total serum IgE level and time interval from the last sting on the decrease of venom-specific IgE over one year is summarized in Table 3. Interaction between the decrease and the total IgE level was significant ($p = 0.011$) by two-way repeated-measures analysis of variance; the total IgE modified the decreasing rate of venom-specific IgE. The decrease was significantly larger in subjects with total serum IgE level ≤ 250 IU/ml than in those > 250 IU/ml. However, the relationship between the decrease and the time interval was not significant ($p > 0.05$); the effect of time interval on the rate of decrease was smaller than that of total IgE. The rates of decrease expressed as a percentage of the difference of venom-specific IgE titer for one year to the initial value were also greater in subjects with total serum IgE level ≤ 250 IU/ml than in those > 250 IU/ml regardless of the time interval from the last sting. In those with a total serum IgE level ≤ 250 IU/ml, the rate of decrease among subjects stung less than 3 years earlier was similar to that in those stung 3 or more years earlier. On the contrary, in those with total serum IgE level > 250 IU/ml, the rate of decrease was unstable and an increase in venom-specific IgE titer was observed even 3 or more years after the last sting.

DISCUSSION

Hymenoptera sensitization can be confirmed by the presence of venom-specific IgE. In the present study, venom-specific IgE was detected in 34.7% of nonallergic subjects. In retrospective surveys, asymptomatic sensitization was pre-

sent in 14-26.8% of nonallergic subjects.^{12,13} Another prospective study about the sensitization risk in nonallergic subjects, found that specific sensitization occurred in 13.7% after a single venom injection and in 31% after two injections.¹¹ Thus, it is obvious that production of specific IgE after *Hymenoptera* stings without manifestation of systemic reactions is quite common. The risk of systemic reactions to subsequent stings in asymptomatic sensitized subjects has not previously been assessed.¹² However, most individuals who have systemic reactions, have no prior *Hymenoptera* sting reactions and are unaware of their potential allergy,¹⁰ suggesting that nonallergic subjects have a risk of systemic reactions with further stings.

The hypothesis that *Hymenoptera* sensitivity is usually transient, was suggested in previous studies to explain the difference in percentage between sensitization and the systemic reactions.^{7,12,14,15} In our studies, the frequency of positive CAP significantly decreased with the time interval since the last sting. A positive CAP was detected in 70% of subjects who were stung less than 3 years earlier and in 21.2% of those stung 3 or more years earlier. Venom-specific IgE titer also decreased with the passage of time. These results agree with previous findings that *Hymenoptera* sensitivity may disappear with the passage of time.^{3,16,17} From our results, in most cases, venom-specific IgE disappeared less than 3 years after the last sting. In previous studies, venom-specific IgE tended to decrease within a few months after *Hymenoptera* stings and persisted as long as 5 years.^{12,18} However, some studies reported

Table 3. Effect of total IgE level and time interval from the last sting on variation in the venom-specific IgE level at one-year follow up in subjects with positive CAP on initial evaluation

Total IgE ^a (IU/ml)	Time interval (years)	N	Venom-specific IgE			
			Titer (UA/ml)		Decrease	
			Initial ^b	One year after ^b	Number ^c	Rate (%) ^{d,e}
≤ 250	<3	6	7.51 (1.82-76.9)	2.67 (0.37-26.8)	5/6	56.8 (-8.0-80.4)
≤ 250	≥3	3	2.58 (1.87-3.17)	1.11 (0.49-2.64)	3/3	51.4 (16.7-73.8)
>250	<3	8	5.25 (1.68-13.8)	3.43 (0.45-19.7)	6/8	16.9 (-108.6-73.2)
>250	≥3	8	2.25 (0.94-14.8)	2.32 (0.65-7.52)	4/8	-22.7 (-190.3-49.2)

Result of two-way repeated-measures analysis of variance^f

Effect of total IgE

Variation by total IgE	ns
Variation for one year	$p = 0.002$
IgE X one year	$p = 0.011$

Effect of time interval

Variation by time interval	ns
Variation for one year	$p = 0.003$
Time interval X one year	ns

^a Values at the initial evaluation were used.

^b Values are geometric means with ranges in the parentheses.

^c The number of subjects whose venom-specific IgE decreased over one year.

^d The rates of decrease are expressed at percentages of the difference in venom-specific IgE titer after one year compared to the initial value.

^e Values are arithmetic means with ranges in parentheses.

^f Values were log-transformed before analyses.

that venom-specific IgE was positive more than 10 years after the last sting.^{4,19} In our study, venom-specific IgE was still detected in 17.6% of subjects who were stung more than 10 years earlier, suggesting that there may be some factors relating to the decline of venom-specific IgE. However, few data are available on factors that may be involved in the production and persistence of venom-specific IgE.¹⁴ Moreover, the factors that regulate the persistence of venom-specific IgE after a sting remain unknown.

In our study, time interval and total serum IgE level were associated with CAP results and total serum IgE levels were significantly elevated in the subjects with positive CAP. Elevated total serum IgE appears to reflect an increased individual IgE immunological response, leading to a higher production of specific IgE to allergens.^{12,22} Moreover, total serum IgE is constituted to a considerable extent by specific IgE in subjects with IgE-mediated disease.^{23, 24} Therefore, we speculated that total serum IgE level is associated with the decline of venom-specific IgE, and that both time interval from the last sting and total serum IgE level are interrelated with the decrease in venom-specific IgE titer.

On evaluating the changes of venom-specific IgE titers for one year, once venom-specific IgE titer fell to an undetectable level, it did not increase again thereafter. Negative CAP findings are used as a criterion for the safe cessation of venom therapy.^{10,25} Based on these results, it is important to follow the venom-specific IgE titer until the level becomes undetectable.

In most subjects with positive CAP, venom-specific IgE titer decreased; during one year follow-up regardless of the time interval since the last sting. However, the venom-specific IgE titer increased in 7 subjects. Three of these seven had been stung less than 3 years earlier and had varied levels of total serum IgE. In comparison, the remaining 4 subjects who had been stung 3 or more years earlier, had total serum IgE levels >250 IU/ml. As we expected at the initial evaluation, an elevated total serum IgE played a role as a modification factor in the decline of venom-specific IgE. Actually, changes in venom-specific IgE titer for one year were quite variable (increase or decrease) in those with elevated total serum IgE level.

Venom-specific IgE titer decreased more in subjects with total serum IgE \leq 250 IU/ml, venom-specific IgE titer decreased fairly progressively regardless of the time interval, compared to that in those > 250 IU/ml. This indicates that the presence of a significant interaction between total serum IgE and the rate of decrease. We suggest that these results reflect the difference in immunological response. Since among those with the elevated total serum IgE have been selected with the passage of time, consequently their decrease in venom-specific IgE titer over one year became unstable. A previous study also reported a difference in the immunological response may exist due to time interval.¹⁴ Based on these results, venom-specific IgE titer was generally strongly influenced by the passage of time, but the difference in total serum IgE level on the decrease in venom-specific IgE titer was more re-

markable when 3 or more years have passed since the sting.

In conclusion, even subjects without allergy to *Hymenoptera* stings showed venom-specific IgE. Venom-specific IgE titer generally decreased with the passage of time and disappeared less than 3 years after the last *Hymenoptera* sting. The total serum IgE level modified the decline of venom-specific IgE, but time interval from the last sting did not. In elevated total IgE level (> 250 IU/ml), the decline of venom-specific IgE tended to be slow.

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