

Evolution of sensitivity to Hymenoptera venom in 200 allergic patients followed for up to 3 years

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Duration of venom immunotherapy still remains questionable, since some patients outgrow their sensitivity. Two hundred venom-allergic patients (4 to 82 years; mean $\pm$ SD, 37.3 ± 14.2 years) who were first observed with systemic reactions (SRs) were investigated yearly for up to 3 years. Among these patients, 72 were allergic to honeybee venom, 83 to yellow jacket venom, and 45 to both yellow jacket and Polistes venom. At each visit patients had skin tests and RAST to venom. All patients had the same semirush protocol and a monthly maintenance dose of 200 μ g. After 1 year of treatment, 19% of bee venom-allergic subjects and 35% of vespid venom-allergic individuals had a negative skin test and RAST to the corresponding venom. The percentage of negatization of tests increased after 3 years to 30.5% in bee venom-allergic patients and to 54% in vespid venom-allergic individuals. This rapid and relatively important negatization of skin tests and RAST may be due to a monthly maintenance dose of 200 μ g; 76 patients experienced a field sting without any SR, two patients had a severe SR, and three patients had a mild SR. Although we tried to define parameters that might predict the abolition of venom sensitivity before starting immunotherapy, we could not find any parameter except venom species. Children and adults behave similarly in terms of evolution of venom sensitization. Mean serum venom-specific IgG levels were significantly lower in patients who had lost their sensitivity. (J ALLERGY CLIN IMMUNOL 1989;84:944-50.)

The duration of VIT is still a matter of discussion, and the question as to whether VIT can be stopped after a certain time is not yet determined. It has been suggested that VIT can be stopped when both skin tests and RAST become negative^{1, 2} or after 5 years of treatment, whatever the results of skin tests or specific IgE.³ Randolph and Reisman,⁴ in their article, stated that patients who had a negative RAST or a low IgE level could stop VIT whatever the results of the skin tests, since they did not experience SR when patients were stung by living Hymenoptera species. Conversely, patients who prematurely stopped VIT when RAST was still positive experienced SR in 25% to 30% of stings in two studies^{5, 6} and in 10% in another study.⁷

Abbreviations used

HB:	Honeybee
HBV:	Honeybee venom
SR:	Systemic reaction
VIT:	Venom immunotherapy
YJV:	Yellow jacket venom
IT:	Immunotherapy

The percentage of patients losing their sensitivity during VIT is poorly determined. In one study, after 3 years of treatment,² 25.8% of 32 adult patients had negative skin tests and RAST. The decrease of positive diagnostic tests appears to occur more frequently in children.⁸⁻¹⁰ Finally, duration of VIT, at least in HBV-allergic children, may be predicted by considering IgE and IgG antibody levels and clinical reactivity in challenged patients. A level of IgE <1 PRU/ml, in association with high IgG titers, might indicate that stopping VIT is possible.¹¹

Since long-term studies, including children and adults, as well as a high number of patients, were not conducted, we decided to study, prospectively, 200 Hymenoptera venom—allergic individuals receiving

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VIT for up to 3 years to estimate the evolution of skin tests and specific IgE. All these patients were followed by yearly investigations, including a questionnaire on sting challenges, skin tests, and serum venom—specific IgE and IgG.

MATERIAL AND METHODS

Patients

Two hundred patients ranging in age from 4 to 82 years (mean $\pm$ SD, 37.3 ± 14.2 years) were included in the study after informed consent. All patients had been first observed with SRs ranging from grade 1 to 4 according to Mueller.¹² Most of the SRs were severe (mean $\pm$ SD: 3.0 ± 1.1). All patients had positive skin tests to one or several of the four venoms tested (HBV, YJV, *Polistes*, and *Vespa crabro*, Stallergènes Laboratories, Fresnes, France). Patients allergic to *V. crabro* venom were not included in the study because their number was too low. Two venom concentrations were tested (0.1 $\mu\text{g}/\text{ml}$ and 1 $\mu\text{g}/\text{ml}$) by intradermal skin test. Patients who had a positive skin test for a concentration of 1 $\mu\text{g}/\text{ml}$ were only desensitized when the RAST (Phadebas RAST, Pharmacia Diagnostics AB, Uppsala, Sweden) to the corresponding venom was positive, because a substantial number of nonallergic individuals have positive skin tests with this venom concentration.^{1, 13, 14} Three patients with a positive skin test for a venom concentration of 0.1 $\mu\text{g}/\text{ml}$ and negative RAST to the corresponding venom received VIT. All other patients had concordant RAST and skin tests.

Methods

Venoms. HB, YJ, and *Polistes* venom were obtained from Stallergènes Laboratories. Venoms are standardized by means of the titration of hyaluronidase and phospholipase A₂, as well as RAST inhibition. HBV and YJV were found to be comparable to venoms of Pharmacia Diagnostics AB.¹⁵

Specific immunotherapy. Specific IT was performed according to a rush or semirush protocol previously published (Table I).^{1, 16} The monthly maintenance dose for each venom was 200 μg .

In case of allergy to vespid venoms, 35% of the patients were treated with both YJV and *Polistes* venom when patients had positive skin tests and RAST to both venoms, since these two species are present in our area.

Skin tests. Intradermal skin tests (0.02 ml) were performed according to the method of Zelesnick et al.¹⁷ with a venom concentration of 0.1 $\mu\text{g}/\text{ml}$. If the test was negative, the venom strength was increased to 1 $\mu\text{g}/\text{ml}$. Since we used several samples of venom during this 7-year study, comparison of simultaneous skin tests with early lots versus recent lots was not possible from the beginning to the end of the study. However, as already stated by Graft et al.^{8, 9} on the basis of enzymatic assays for phospholipase A₂ and hyaluronidase, there is a similar lot-to-lot potency of venoms, and we have tested by skin test end point several lots of venoms and found them to be similar. Moreover, the reproducibility of venom batches appears to be far greater than that of skin testing; therefore, skin tests performed on

TABLE I. Immunotherapy schedule*

Day	μg
1	0.001
	0.01
	0.1
2	1
	5
3	10
	20
8	20
	30
9	50
	50
15	100
28	100
	100
35	100
	200

*Monthly maintenance dose, 200 μg .

several occasions are unlikely to be greatly modified by the strength of venom lots.

Serum-specific IgE antibodies. Serum-specific IgE was titrated by means of the classic Phadebas RAST (Pharmacia Diagnostics AB). Since we have already found that venoms from Stallergènes and Pharmacia Laboratories were comparable,¹⁵ we did not use specific disks prepared with venoms from Stallergènes Laboratories. Results were expressed in semiquantitative classes according to the Pharmacia scoring system. Although it would have been desirable to test initial sera and followed sera at the same time, this was not feasible because we followed a large number of patients during a period of 3 years. Moreover (1) it is not certain that sera stored for up to 3 years do not lose some IgE molecules, and (2) we did not want to assess the evolution of specific IgE but to determine if specific IgE was still present in the serum of the patient at different times after initiation of VIT.

Serum-specific IgG antibodies. Serum-specific IgG antibodies were titrated by means of a double-antibody radioimmunoassay with a method previously described in detail.^{18, 19} Briefly, venom allergens (HBV, Sigma Chemical Co., St. Louis, Mo., and YJV from Vespa Laboratories [Spring Mills, Pa.], kindly provided by Pharmacia Diagnostics) are labeled with ¹²⁵I in phosphate buffer and chloramine-T. The reaction is stopped by sodium metabisulfite, and unbound ¹²⁵I is separated from iodinated allergens by gel filtration on column Sephadex G-25. The entire radioactive (void volume) peak was pooled, aliquoted, and stored at -80°C until use. The yield of iodination is $9.8 \pm 1.6\%$ for YJV and $18.8 \pm 3.3\%$ for HBV ($n = 6$, with three different batches of venom), and the average specific activities were, respectively, of 37.0 kBq/ μg and 70.3 kBq/ μg . The titration of venom IgG was performed by incubating patient's serum with iodinated venom allergens and precipitating antigen-antibody complexes by goat antihuman IgG in presence of 4% polyethylene glycol, 6000. Inhibition studies have demonstrated the specificity of the test, and

TABLE II. Results of venom-specific IgE and IgG in the two patients who experienced an SR during field sting

	Patient 1		Patient 2	
	IgE (PRU/ml)	IgG (%)	IgE (PRU/ml)	IgG (%)
Values				
Baseline	>17.5	ND	>17.5	5
Pre-SR	8.0	35	11.0	58
Post-SR	>17.5	13	16.0	22

ND, Not done.

TABLE III. Demographic parameters of patients allergic to HBV and vespid venoms according to their sensitivity after immunotherapy

Venom species sensitivity	HBV		<i>p</i>	Vespid venom		<i>p</i>
	Present	Absent		Present	Absent	
No. of patients	50	22	56	72		
Age (yr)	37.2 ± 14.2	32.4 ± 18.8	NS	40.9 ± 14.5	38.3 ± 17.2	NS
Last sting (yr)	2.9 ± 2.3	2.0 ± 3.7	NS	2.1 ± 2.5	1.6 ± 1.9	NS
No. of stings	4.2 ± 2.6	4.6 ± 3.2	NS	3.8 ± 2.9	3.3 ± 3.3	NS
No. of SRs	2.2 ± 0.8	1.8 ± 0.9	NS	2.5 ± 4.1	1.9 ± 1.4	NS
Severity of SR	3.1 ± 1.3	3.2 ± 1.5	NS	2.9 ± 1.3	3.1 ± 1.4	NS
RAST before IT	2.6 ± 1.5	1.9 ± 1.3	<0.02	1.5 ± 1.3	1.5 ± 1.1	NS

NS, Not significant.

Statistical analysis by Student's *t* test.

serum containing elevated venom IgE titers did not influence the results. Inter- or intra-assay coefficients of variation ranged from 4.7% to 15.0%.¹⁶ Results are expressed in percent of maximum binding capacity that is calculated by dividing the net counts per minute bound by the maximum net binding of ¹²⁵I-labeled venom observed in IgG antivenom antibody excess.

Follow-up of the patients. At each visit patients filled in a questionnaire, including reactions that might have occurred during VIT, and eventual field stings. The IT schedule was checked.

Design of the study

All patients had identical investigations, including clinical history, skin tests, and RAST before VIT, and every year for up to 3 years. Sensitivity was considered to be lost when skin tests and RAST became negative. When sensitivity was lost, VIT was stopped.

At the end of the study or when sensitivity had been lost, 125 patients had a titration of serum-specific IgG antibodies. IgG antibodies were only titrated in some patients after IT, since this technique was not available when we started the survey. We initially assayed IgG with a different method of titration that was found to be less accurate.¹⁸

The whole study lasted 7 years, from 1981 to 1987.

Statistical analysis

Although the study presented herein is essentially descriptive, statistical analysis was performed by means of parametric tests (Student's *t* test) to compare the mean IgE and IgG antibody levels in the sensitized and desensitized patients.

RESULTS

SRs observed in patients

Seventy-two patients were allergic to HBV, 83 to YJV, and 45 to both YJV and *Polistes* venom. One patient allergic to HBV had to stop VIT 2 years after its onset because he suddenly experienced an SR after each injection, even when doses were decreased to 10 µg. Serum-specific IgE levels titrated a week after the last injection were extremely high (105 PRU/ml; result was obtained after dilution of serum), and serum IgG antibodies were very low (15%).

Seventy-six patients experienced a field sting and were apparently stung by the same Hymenoptera species that elicited the initial SR. Three had mild SR (grade 1, according to Mueller¹²), and two other patients experienced severe SR, requiring epinephrine. One of these patients was allergic to HBV, and the

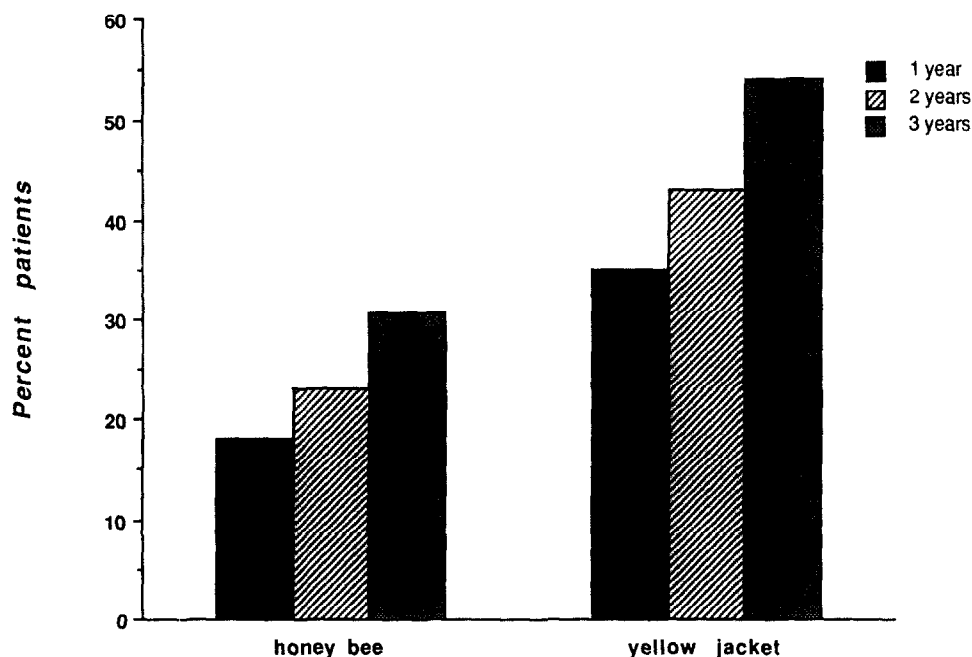


FIG. 1. Percentage of patients with negative skin test and RAST according to the duration of immunotherapy.

other patient to YJV. In both cases, the levels of serum-specific IgE measured from 2 to 4 weeks after the SR were increased in comparison to previous investigations, and the levels of serum-specific IgG were decreased (Table II).

Evolution of sensitivity to venom

The percentage of patients who had lost their sensitivity, as defined by negative skin tests and RAST, is presented in Fig. 1. Only 30.5% of HBV-allergic individuals had lost their sensitivity after 3 years of treatment. In contrast, 35.5% of vespid venom-allergic individuals had lost their sensitivity after 1 year of treatment, and this percentage increased to 54% after 3 years of treatment. There was no difference between patients treated with YJV and those treated with YJV and *Polistes* venom; therefore, results of YJV and mixed vespid venom-allergic individuals were averaged. All three patients with a negative RAST before VIT had negative skin tests after 1 to 3 years of treatment. Eight patients who were both allergic to HBV and YJV retained the sensitivity to one venom (HBV, six cases; YJV, two cases) and lost the sensitivity to the other venom.

Characteristics of patients before IT are presented in Table III. Patients who had lost their sensitivity are comparable in terms of age, severity, or number of SRs, and gap between the last reaction and VIT with patients in whom sensitivity was retained. RAST be-

fore IT was significantly greater in patients retaining sensitivity to HBV than in patients who have lost the sensitivity. However, for vespid venoms, we could not find any difference.

The mean duration of VIT in patients who had lost their sensitivity was 2.4 ± 1.2 years in HBV-allergic patients and 2.3 ± 1.3 years in vespid venom-allergic patients.

Children did not lose their sensitivity quicker than adults (Figs. 2 and 3). In YJV allergy, patients older than 30 years tend to be more apt to lose their sensitivity than younger patients, but the difference is not significant.

Serum venom-specific IgG

IgG levels were significantly higher in patients who retained their sensitivity than in patients who had lost sensitivity, whatever venom was injected (Fig. 4).

DISCUSSION

The present study demonstrates that a large number of patients lose their sensitivity after a shorter period of time than expected and confirms previous surveys indicating that VIT is effective. Patients allergic to HBV retain significantly more sensitivity than patients allergic to vespid venoms. Serum venom-specific IgG is significantly lower in patients having lost their sensitivity than in patients who retained sensitivity.

Many studies¹ have previously demonstrated that

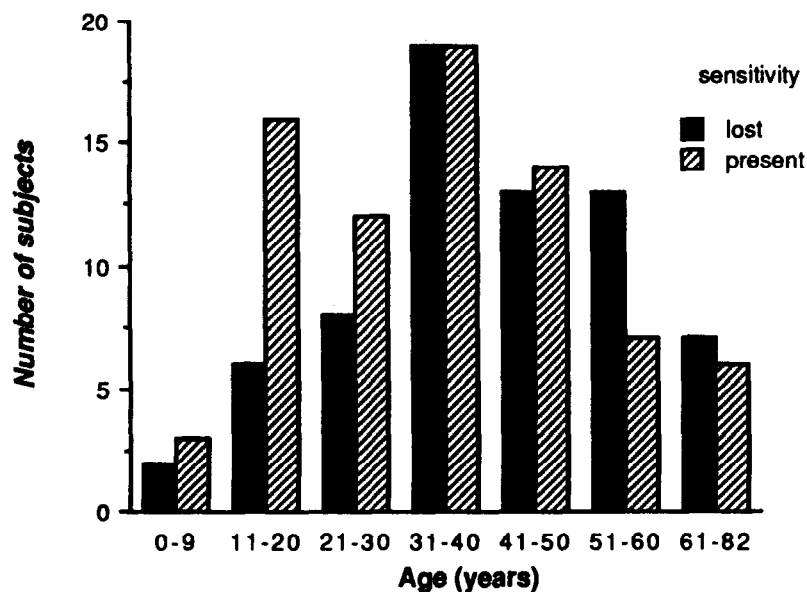


FIG. 2. Sensitivity to venom according to the age of the patients in subjects allergic to HBV.

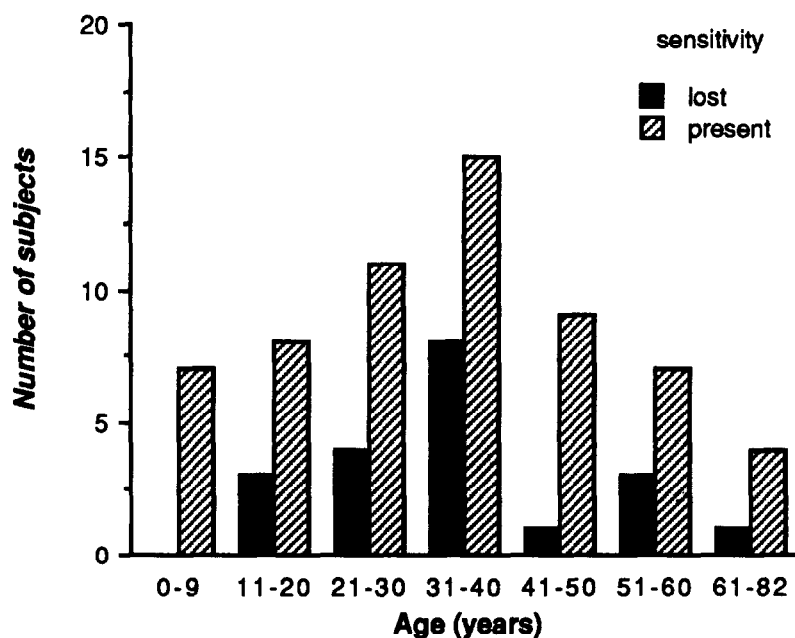


FIG. 3. Sensitivity to venom according to the age of the patients in subjects allergic to vespid venoms (YJV and/or *Polistes*).

VIT is effective in >90% of patients treated, and this study confirms such results, although the stinging insect was not often entomologically identified. There were too few SRs experienced by venom-treated patients to assess the predictive value of venom-specific IgE or IgG antibody measurements, but it appears that all patients with SR had an increased IgE level and a low IgG level shortly after the SR. The present study

also indicates that VIT had to be stopped in one patient because of severe SRs during VIT. Such a case appears to be extremely rare but does occur and might benefit from passive immunization,^{15, 20} but we did not use passive immunization because fractionated IgG were not available when these reactions appeared.

This study was not to prove that some tests have any predictive value on the efficacy of VIT nor that

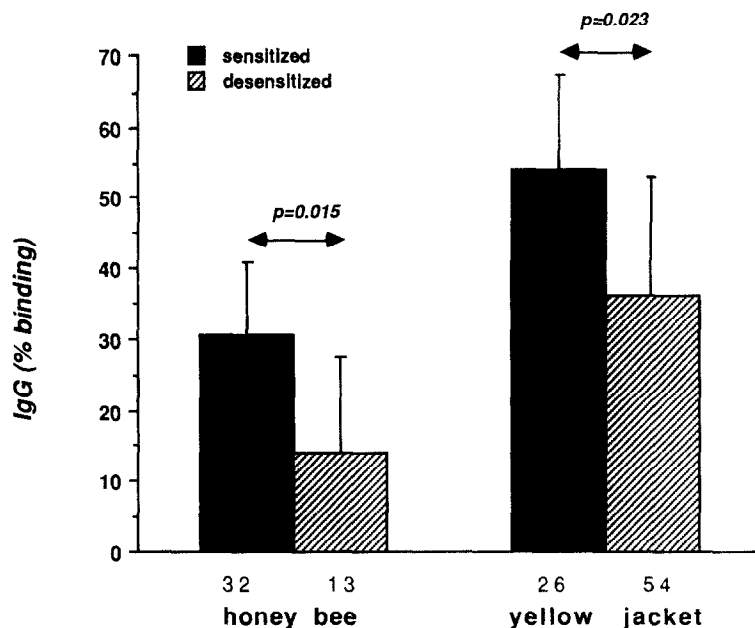


FIG. 4. Mean serum venom-specific IgG in patients who had lost or retained their sensitivity to venom. Numbers indicate the number of patients tested in each group.

only patients with negative skin tests and RAST are protected when VIT is stopped but to determine the percentage of patients who lose their sensitivity to venom during VIT. The sensitivity of the patients was considered to be lost when both the intradermal skin test for a venom strength of 1 µg/ml and the RAST became negative. Serum-specific IgE was measured by Phadebas RAST. We did not titrate all IgE levels of a patient in one experiment series because (1) it was not easy to do on this large a number of patients and (2) we did not want to prospectively evaluate IgE levels but to determine whether patients had a positive RAST before IT and at the end of the trial. Negativation of RAST is not likely to be due to RAST interference,²¹ since mean serum IgG levels were lower in patients without sensitivity than in patients who retained it. In the study of Thurnheer et al.² the rate of negativation of the RAST is similar to rate observed in our study, whereas in both studies of Graft et al.^{8,9} negativation of specific IgE was exceptional. This discrepancy may be due to a more precise IgE test used in the American study or, alternatively, to a larger amount of allergen injected in the European studies.

The rate of negativation of skin tests is higher from other studies of patients followed for 3 to 5 years under maintenance VIT, since it ranges from 27.6% in the study of Thurnheer et al.² to 45% in the first study of Graft et al.⁸ and 54% in our study for vespid venoms. This rather high rate of negativation of skin tests should not be unexpected, since a high proportion

of beekeepers stung by >200 HB a year lose their sensitivity to HBV skin tests and HBV RAST, whereas beekeepers who are stung by fewer insects often retain their sensitivity to HBV.²² The follow-up of patients allergic to vespid venoms demonstrates that they have lost their sensitivity at a much quicker rate than expected, perhaps because we used a higher maintenance dose than is usually recommended. This rapid desensitization rate favors yearly investigations to stop possibly VIT in patients with negative skin tests and RAST. For HBV, the rate of negativation of skin tests and RAST is twice the rate of that in the study of Thurnheer et al.,² possibly because we used a higher maintenance dose. We did not find that children lose their sensitivity at a higher rate than adults, as it was suggested in previous studies.⁸⁻¹⁰ As has already been observed in other studies,^{2,8,9} we found that the loss of sensitivity for one venom does not preclude that the patient allergic to many venoms had lost sensitivity to all of them. Finally, to our surprise, there was no parameter that may predict the evolution of individual patient's sensitivity.

The natural history of untreated allergic patients indicates that many subjects spontaneously lose their sensitivity. Among the 29 allergic patients followed for 6 to 22 years by Savliwala and Reisman,²³ 24% had a negative skin test and RAST, and 44% had a decreased skin test reactivity. We do not have any indication of the natural history of patients of our area, but it may be anticipated that it is similar to other literature.^{1,24}

Venom-specific IgG is still a matter of debate. Although it is commonly accepted that patients having a high IgG titer within a year after the initiation of VIT are likely to be protected, there are many protected patients with low titers, and, in contrast, many other patients that are not protected with high IgG levels.¹ After a few years of VIT, the situation is even more complex, and the value of IgG is unknown. Some investigators confirmed the importance of high IgG levels in the protection of patients,²⁵ whereas other investigators found that many protected patients had levels close to pretreatment values.²⁶ In the present study, mean IgG levels were significantly lower in patients with negative skin tests and RAST. Graft et al.⁸ found that mean YJV IgG was slightly lower in children with negative skin tests, whereas HBV IgG was slightly higher in children with negative skin tests. In fact, these studies do not elicit any definite conclusion on the role of venom IgG in long-term IT.

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