
Comparison of 2 maintenance doses (100 μ g vs 200 μ g) in Hymenoptera venom immunotherapy: influence of the maintenance dose on the immunologic response

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Background: In Hymenoptera venom immunotherapy, the maintenance dose is usually 100 μ g. However, persistent systemic reactions to sting challenges could be treated by an increase in the maintenance dose to 200 μ g with success, suggesting greater efficiency.

Objective: To compare the effects of 2 monthly maintenance doses (100 μ g vs 200 μ g) on skin test sensitivity and venom specific IgE antibody levels.

Methods: Twenty-two patients receiving *Vespula* venom immunotherapy were enrolled in this retrospective study. After rush therapy, the 100- μ g maintenance dose initially administered was maintained (group 1, n = 13) or was increased to 200 μ g (group 2, n = 9).

Results: Levels of venom specific IgE antibody and skin test results measured before the onset of immunotherapy were comparable in both groups. Unlike in group 1, a maintenance dose of 200 μ g resulted in significant decreases in venom specific IgE antibody levels and skin test sensitivity.

Conclusions: Increasing the monthly maintenance dose to 200 μ g results in a greater degree of change in venom specific IgE antibody levels and skin test sensitivity than when maintaining a 100- μ g dose. Our data strengthen those of previous clinical studies showing the usefulness of a 200- μ g maintenance dose in the case of clinical failure of a 100- μ g dose.

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INTRODUCTION

In patients with Hymenoptera venom allergy, venom immunotherapy (VIT) is considered to be the treatment of choice, with a protection rate ranging from 80% to 98%.¹ This therapy is inconvenient for patients because the maintenance immunotherapy is administered at 4- to 6-week intervals and is maintained for up to 3 to 5 years.^{2,3} Thus, some researchers^{4–6} have suggested an increase in the maintenance interval to every 6 to 8 weeks and even every 3 months. Such intervals result in a similar protective activity of immunotherapy against repeated stings.^{5,6}

The maintenance dose is usually 100 μ g every month,^{3,7–12} and in most previous studies,^{3,8–12} this conventional maintenance dose was highly effective. However, a wide range of patients (2.4%–20.4%) may not be protected by immunotherapy.^{13,14} In patients not protected while receiving a dose of 100 μ g, an increase in the maintenance dose to 200 μ g can provide full protection, with no reaction to challenge sting.^{12,15–17} These results suggested that a 200- μ g maintenance dose has greater efficiency. Nevertheless, to the best of our knowledge, with respect to the effects on venom specific

IgE levels and skin test reactivity, a protocol that includes a progressive increase in the maintenance dose to 200 μ g has not previously been compared with a protocol that maintains the maintenance dose at 100 μ g.

In the Respiratory Department at the University Hospital in Amiens, France, we previously used both maintenance doses in patients with yellow jacket venom allergy. Thus, we conducted a retrospective study to analyze the immunologic effects of an increase in the monthly maintenance dose from 100 μ g to 200 μ g. Changes in skin test reactivity and serum venom specific IgE levels during VIT were studied for up to 2 years and were compared with those of patients taking a standard maintenance dose of 100 μ g. We hypothesize that higher maintenance doses will allow a greater immunologic response in terms of skin sensitivity and venom specific IgE antibody levels.

MATERIALS AND METHODS

Population

Patients enrolled in this retrospective study had a history of systemic anaphylactic reactions to yellow jacket (*Vespula* species) stings. The severity of systemic reactions ranged from grade I to grade IV according to the classification of Mueller.¹⁸ All patients undergoing Hymenoptera VIT with *Vespula* venom had positive skin test results or elevated levels of serum venom specific IgE. Patients were excluded

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from VIT if they were not compliant or in the case of pregnancy, β -blocker or angiotensin-converting enzyme inhibitor therapy, mastocytosis, or serious medical or surgical illness.

Immunotherapy Protocol

The initial rush therapy was performed during a 24-hour hospitalization and corresponded to an incremental phase during which the venom dose was progressively increased. During this hospitalization, patients received 13 injections (lyophilized *Vespula vulgaris* preparation), which were given subcutaneously at 30-minute intervals. The initial concentration was 0.0001 $\mu\text{g/mL}$ (0.1 mL) and was progressively increased to 100 $\mu\text{g/mL}$ (1 mL) 6 hours later. The initial period was followed by 2 injections of 60 μg each on day 15 and 1 injection of 100 μg on days 30 and 60 (Table 1).

Then, with respect to the maintenance protocol, patients usually received a venom dose of 100 μg once a month for 1 year (group 1). Some patients received a venom dose of 100 μg once a month for 1 year and then a progressive increase in the maintenance dose from 100 to 200 μg per month during a 5-month period (group 2) (Fig 1). The group assignment was made by the physician.

Immunologic Tests

In all patients, similar procedures, including skin tests and dosages of serum venom specific IgE, were performed before the beginning of immunotherapy (T_0) and 2 years after the first injection of a maintenance dose of 100 μg (T_1). A positive skin test reaction corresponded to a wheal of at least 5 mm in diameter with erythema after 15 minutes. Four venom (*Vespula* species) concentrations were tested (0.001, 0.01, 0.1, and 1 $\mu\text{g/mL}$) by intradermal skin test. A volume of 0.02 mL of each solution was injected. When a test result was negative for a concentration, the venom strength was in-

creased to the superior concentration. The lowest concentration that resulted in a positive reaction was considered to be the end point concentration. Venom specific IgE antibody levels were measured using the Pharmacia CAP system (Pharmacia, Uppsala, Sweden). This system includes a solid phase with a polymer enclosed in a capsule and a combination of monoclonal and polyclonal anti-IgE.

Statistical Analysis

Data are expressed as mean $\pm$ SD. In each group, skin test sensitivity and serum venom specific IgE levels measured before (T_0) and during (T_1) maintenance immunotherapy were compared using a nonparametric Wilcoxon test, whereas comparisons between groups 1 and 2 were performed using a Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

RESULTS

Twenty-two patients (15 men and 7 women) receiving *Vespula* VIT were included in this study. The mean patient age was 39.1 ± 12.4 years (range, 22–66 years). The severity of the systemic reaction to insect sting according to the grading of Mueller was as follows: grade I in 7 patients, grade II in 2 patients, grade III in 5 patients, and grade IV in 8 patients. Group 1 consisted of 13 patients who received a 100- μg maintenance dose every 4 weeks, and group 2 consisted of 9 patients in whom the monthly maintenance dose was increased to 200 μg . No significant differences in age, sex, and severity of the initial reaction to the yellow jacket sting were observed between groups.

The interval between administration of the first 100- μg maintenance dose to the second measurement of venom specific IgE antibodies and repeated skin testing was 21.6 ± 1.7 months for group 1 and 22.6 ± 0.5 months for group 2 ($P = .34$). Therefore, for this period, the cumulative dose of wasp venom was higher in group 2 ($3,411 \pm 237 \mu\text{g}$) than in group 1 ($2,162 \pm 171 \mu\text{g}$) ($P < .001$).

Regarding immunologic tests, no significant differences in initial skin test results were noted between groups ($P = .14$). Furthermore, the levels of venom specific IgE antibodies measured before the onset of VIT (T_0) were comparable between groups 1 and 2 ($18.4 \pm 30.9 \text{ kUI/L}$ and $15.6 \pm 19 \text{ kUI/L}$). In patients who received a maintenance dose of 200 μg , significant decreases in venom specific IgE antibody levels were noted between T_0 and T_1 ($P < .01$), and positive skin test results were achieved with significantly higher venom concentrations ($P < .03$). In contrast, no significant changes in skin test sensitivity and venom specific IgE antibody levels were observed during the same period in group 1 (Fig 2). Therefore, the higher maintenance dose of VIT resulted in greater changes in immunologic test results than conventional dosing.

During rush immunotherapy, systemic reactions were observed in 2 patients. The severity of these reactions was grade I in 1 patient and grade IV in 1 patient according to the scale

Table 1. Rush Immunotherapy Protocol With Progressive Increases in Venom Doses for the First 2 Months

Day	Hour	Concentration, $\mu\text{g/L}$	Volume, mL	Dose, μg
1	0	0.0001	0.1	0.00001
	0.5	0.001	0.1	0.0001
	1	0.01	0.1	0.001
	1.5	0.1	0.1	0.01
	2	1	0.1	0.1
	2.5	1	0.5	0.5
	3	10	0.1	1
	3.5	10	0.5	5
	4	100	0.1	10
	4.5	100	0.2	20
	5	100	0.3	30
	5.5	100	0.6	60
	6	100	1	100
15	0	100	0.6	60
	0.5	100	0.6	60
30	NA	100	1	100
60	NA	100	1	100

Abbreviation: NA, not applicable.

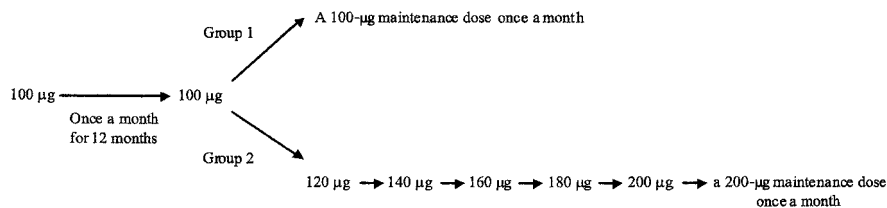


Figure 1. Protocols of maintenance immunotherapy in groups 1 and 2.

of Mueller. No severe systemic reactions were observed during maintenance immunotherapy in either group.

DISCUSSION

The results of this study demonstrate that in contrast to a dose of 100 μg , an increase in the monthly maintenance dose to 200 μg leads to a significant decrease in venom specific IgE antibodies and skin sensitivity to yellow jacket venom. Some methodological aspects of this study merit consideration. The number of patients was small, but our population can be considered homogeneous. In fact, we only considered patients who had systemic anaphylactic reactions to a yellow jacket (*Vespula* species) sting and received a similar VIT protocol, with immunologic tests performed before the onset of treatment and after 2 years of maintenance immunotherapy. In our study, we analyzed only the immune response and not the clinical efficiency with repeated sting challenge. We can only postulate potential clinical implications from these immunologic differences observed between both maintenance doses, and no valid clinical conclusions based on these results could be established. The immunologic effects of Hymenoptera VIT were evaluated by skin test sensitivity and serum venom specific IgE antibody levels and not by serum venom IgG antibody levels. Measurements of IgG were not used because studies^{9,16,17,19–24} designed to identify an association between IgG levels and clinical protection have demonstrated contrasting results. For the measurement of IgE antibodies, a CAP system was used. This system seems to be more sensitive than the traditional IgE radioallergosorbent test (RAST) without a significant decrease in specificity.²⁵

The efficiency of VIT in patients allergic to honeybee or yellow jacket venom is widely demonstrated. As early as 1977, Light et al²⁶ demonstrated that treatment using bee venoms seemed to constitute an effective therapy. In contrast to whole-body insect extracts, bee venom therapy leads to an increase in venom specific IgG antibody levels, whereas venom specific IgE antibody levels decrease. Regarding maintenance therapy, most researchers^{3,4,7–12,27} recommend a maintenance dose of 100 μg with a 4- to 6-week interval and even a 12-week interval between 2 injections. This maintenance dose provides excellent protection during and after completion of a full course of VIT for most patients. Lower venom doses, such as 50 μg , have been administered previously. Golden et al²⁷ thus showed a greater response to VIT with a maintenance dose of 100 μg than with 50 μg , suggesting that the response was dose dependent. However, a

maintenance dose of 50 μg could be sufficient in most patients. Reisman and Livingston²⁸ observed only 3 systemic reactions after 258 repeated stings in patients treated with a 50- μg maintenance dose. Inversely, higher maintenance venom doses have been administered with great results either directly¹⁶ or in the case of failure with a maintenance dose of 100 μg .^{12,15} Using a maintenance dose of 200 μg , Bousquet et al¹⁶ reported mild or severe systemic reactions in only 5 of 76 patients who had experienced a field sting. Rueff et al¹² showed that an increase in the maintenance venom dose to 200 μg could result in full protection in patients with systemic reactions to challenge sting despite VIT using a 100- μg maintenance dose. In the same way, Muller et al¹⁵ used a maintenance dose of 100 μg in patients allergic to honeybee venom. This dose was increased to 200 μg when the increase in venom specific IgG was less than 3 $\mu\text{g}/\text{mL}$. The difference in terms of systemic reactions to challenge sting was higher with a maintenance dose of 100 μg than with 200 μg without reaching significance.

In this study, we explored immunologic variables only (venom specific IgE antibody levels and skin test sensitivity). Therefore, it is difficult to establish clinical conclusions based on these immunologic results. Earlier studies simultaneously evaluated clinical and immunologic data. In patients with a history of insect sting-induced anaphylaxis, it was thus previously demonstrated that neither skin test results nor serum levels of venom specific IgE and IgG4 could predict the clinical response and the severity of systemic reactions to yellow jacket or honeybee sting challenge.^{21,23,24} Lerch and Muller³ compared changes in immunologic data observed with a 100- μg maintenance dose of *Vespula* venom in patients with relapse and continued protection. No significant differences in skin test results or in venom specific IgE antibody levels between groups were observed on discontinuation of VIT. However, other previous studies have suggested that these immunologic tests were helpful. Golden et al²⁹ thus reported that venom specific IgE antibody levels before sting challenges were higher in patients with systemic reactions to stings than in nonreactive patients. Van Halteren et al³⁰ also showed that the relapse rate was significantly higher in patients with a high level of yellow jacket specific IgE antibodies after immunotherapy than in patients with a lower level. Regarding immunotherapy with honeybee venom, Muller et al¹¹ noted a tendency toward lower skin test sensitivity and a lower venom specific IgE level before discontinuation of immunotherapy and before sting challenges

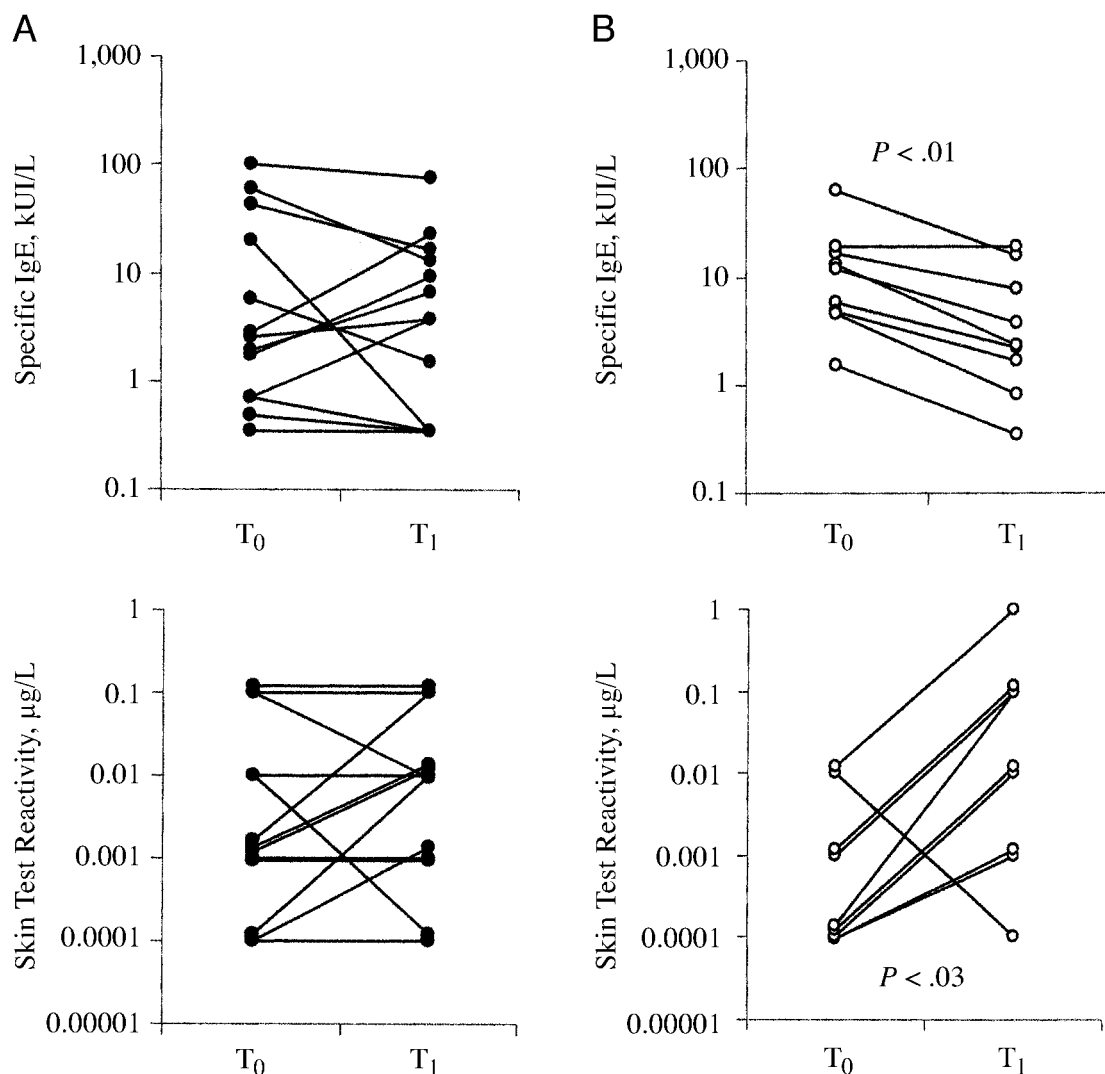


Figure 2. Changes in specific IgE antibody levels and skin test sensitivity from the time of initial immunologic tests, before the onset of venom immunotherapy (T₀), to 2 years after the first injection of a maintenance dose of 100 µg (T₁) in group 1 (A) and group 2 (B). For skin tests, the concentration corresponds to the lowest concentration that resulted in a positive reaction.

in patients with lasting protection than in relapsed patients. Finally, a nondetectable venom specific IgE antibody level associated with a skin test end point concentration of 10^{-3} g/L or more would be in favor of great protection.^{3,11}

It has been established that VIT can lead to a progressive decrease in venom specific IgE antibody levels and in skin test sensitivity.^{3,11,15,16,30} However, changes in venom specific IgE antibody levels and in skin test sensitivity in accordance with the maintenance dose have been poorly studied. Rueff et al,¹² who used a maintenance venom dose of 100 µg and then 200 µg in some patients, did not perform skin tests and RASTs. In the study by Muller et al,¹⁵ no comparison in terms of immunologic results was reported between patients receiving a 100- or 200-µg maintenance dose. Nevertheless, this

relation between changes in immunologic results and the maintenance dose can be suspected. Indeed, Bousquet et al¹⁶ reported a rapid negatization of negative skin test results and RAST results when using a maintenance dose of 200 µg and thus suggested that this result could be due to the higher maintenance dose (200 µg). At last, Golden et al²⁷ measured levels of venom specific IgG that were significantly lower with a 50-µg maintenance dose than with 100 µg.

In the present study, changes in immunologic test results in accordance with the maintenance dose were analyzed. The effects of increasing the maintenance venom dose to 200 µg (after 1 year of 100 µg monthly) were thus compared with standard maintenance therapy using 100 µg per month. We noted that the maintenance dose of 200 µg led to a greater

change in the immune response than a conventional dose of 100 μg . Thus, sensitization decreases significantly with a 200- μg maintenance dose: changes in venom specific IgE antibody levels and skin test sensitivity seemed to be more homogeneous with a significant decrease for the group as a whole. In contrast, in group 1 (100 μg), the immune response during VIT varied widely from patient to patient in terms of venom specific IgE antibody levels and skin test results.

In summary, the results of this study demonstrate that compared with a dose of 100 μg , a maintenance dose of 200 μg provides greater hyposensitization for the same period of treatment. It can thus be postulated that an increase in the maintenance dose to 200 μg might allow a reduction in the duration of VIT. However, it is conjectural whether changes in skin test sensitivity and venom specific IgE antibody levels would be correlated with better treatment outcomes than conventional VIT, which is effective for most patients. Nevertheless, these immunologic data are in agreement with clinical results previously reported in the literature such that a monthly maintenance dose of 200 μg can be useful and effective in the case of clinical failure of a monthly dose of 100 μg .

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