

Evolution of lymphocyte transformation to wasp venom antigen during immunotherapy for wasp venom anaphylaxis

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Summary

Background Venom immunotherapy (VIT) has proven to be safe and effective in wasp venom anaphylaxis. However, there are no good parameters to indicate when to stop venom immunotherapy.

Objective To evaluate the relationship of the lymphocyte transformation test (LTT) to history and specific IgE determination, and to address the time course of lymphocyte transformation responses to wasp (*Vespula*) venom during VIT and the possible utility of LTT to determine the duration of therapy.

Methods Peripheral blood mononuclear cells (PBMCs) of 18 individuals with a history of wasp sting anaphylaxis and a positive serum-venom-specific IgE, were stimulated with wasp venom before immunotherapy, at the end of a 5-day semi-rush immunotherapy and at 24 months during venom immunotherapy. Results, expressed as stimulation index (SI), were compared with the SI in seven asymptomatic stung controls.

Results In controls the median (minimum–maximum) of the SI were 2.39 (0.52–3.39) before therapy and 2.39 (1.12–6.02) when repeated after 24 months. For patients the median (minimum–maximum) of the SI were 10.13 (1.19–44.88) before immunotherapy (d0), 2.73 (0.67–12.03) at the end of the build-up immunotherapy (d5) and 4.21 (0.88–14.66) at the end of 24 months of maintenance therapy (m24). The proliferation responses in vespid-allergic patients were significantly higher than in stung controls ($P = 0.006$) but only 13/18 patients showed a positive LTT result before the start of immunotherapy (sensitivity of the LTT 72%). When the LTT was repeated after a 5 day build-up hyposensitization course the SI significantly dropped as compared to the pre-treatment levels ($P = 0.002$). The SI of the LTT was negative in eight out of 18 patients at 24 months and the median values were significantly lower than before therapy ($P = 0.03$).

Conclusions Although, in the absence of sting challenge data it is not possible to draw conclusions about the predictive value of the LTT, our data may suggest that abolition of the LTT during VIT might indicate clinical insensitivity. Further studies, comparing the results of sting challenges, with the results of lymphocyte transformation will be necessary in order to evaluate the role of LTT in stopping immunotherapy.

Keywords: hymenoptera allergy, wasp venom, immunotherapy, lymphocyte transformation test

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Introduction

Properly administered wasp venom immunotherapy (VIT) has proven to be a safe and effective treatment, providing

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≈ 98% protection to individuals at potential risk for subsequent wasp-sting anaphylaxis [1–3]. However, some issues, such as when VIT can be safely discontinued, remain unresolved. Abolition of serum venom-specific IgE [6–9], loss of venom skin test reactivity, and VIT for a finite period of 3–5 years, irrespective the persistence of positive

specific IgE or skin tests [8–13], have been proposed as possible guidelines for stopping VIT [6–13]. In a number of studies, recently reviewed by Ruëff et al. [14], sting challenge was used to evaluate therapeutic efficacy of immunotherapy. However, due to practical and ethical considerations, sting challenge has not widely been accepted as the method of choice for evaluating treatment results. The present study was designed to evaluate the LTT to wasp venom as a possible additional parameter between documented vespid-allergic individuals (positive history and specific IgE) and asymptomatic stung controls (negative history and specific IgE), to address the evolution of the LTT during VIT, and to investigate the possible utility of LTT to determine the duration of therapy.

Methods

Subjects and immunotherapy protocol

Eighteen patients (six men and 12 women) aged from 15 to 67 years who had exhibited systemic anaphylaxis to yellow jacket stings (*Vespula vulgaris*) with a positive serum venom-specific IgE were enrolled. All of them were treated according to the following protocol with wasp venom (Pharmalgen-ALK Wasp venom, Kabi-Pharmacia, Brussels, Belgium). Each patient was initially administered a dose of 1 pg of venom. The dose was progressively increased over 5 days. On the fifth day of build-up therapy 80 and 100 µg were injected. A maintenance dose (100 µg per injection) was administered at days 12, 26, 37 and once every 4 weeks during the first year, and subsequently every 6 weeks. The patients were followed up for 24 months. Seven healthy volunteers (three men and four women) aged from 23 and 52 years with a previous wasp sting without symptoms of anaphylaxis and with a negative serum venom-specific IgE (<0.35 kU/L), served as non-symptomatic stung controls. All patients and controls gave informed consent for the study.

Lymphocyte transformation test

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation (density 1.077 g/ml). 1×10^5 lymphocytes in 150 µL serum free AIM-V medium (Gibco Life Technologies, Gent, Belgium) were stimulated in quadruplicate with 20 µL (10 µg/mL) *Vespula* venom (Pharmalgen-ALK Wasp venom, Kabi-Pharmacia), or medium as negative control in 96-well round-bottomed microtiter plates (Falcon Becton Dickinson Benelux, Erembodegem, Belgium). The dose of 10 µg/mL of *Vespula* venom was chosen as giving the best stimulation after

initial testing with doses of 1, 10 and 100 µg/mL. The plates were incubated at 37°C for 6 days in a humidified 5% CO₂ incubator (Jouan, Saint Herblain, France). Cells were pulsed for an additional 24 h with 20 µL of [methyl-³H]-thymidine (1 µCi/well; specific activity 25 mCi/mmol, Amersham Life Sciences, Ghent, Belgium). The cells were harvested with a multiple automatic cell harvester (Automash 2000, Dynatech Laboratories, Chantilly, VA, USA) on glass fibre filter paper (MN GF-6, Machery-Nagel, Düren, Germany). The incorporated radioactivity was measured with a liquid scintillation counter (Packard Tricarb, Packard Instruments, Meriden, CT, USA). Results were expressed as stimulation index (SI), i.e. the counts per minute obtained with the antigen stimulation, divided by the counts per minute, obtained with the control experiment without antigen. The highest SI value of the controls was used as the limit between clinically positive and negative samples. Blood samples were obtained on day 0 (d0) before start of immunotherapy, repeated at day 5 (d5) at the end of the semirush build-up course and at 24 months of maintenance immunotherapy. In the control group, LTT was performed on d0 and repeated after 24 months.

Venom-specific IgE

Quantification of serum wasp venom-specific IgE was performed by a radioactive CAP assay (Kabi-Pharmacia CAP System, Brussels, Belgium). Results were expressed as international kilo units per liter (kU/L). A serum concentration less than 0.35 kU/L was considered as negative.

Statistical analysis

Results were expressed as the median with corresponding minimum and maximum. The Mann-Whitney *U*-test and Wilcoxon matched paired signed-ranks test were applied where appropriate. The Chi-square test was used to compare frequencies. Differences were considered significant at a $P < 0.05$.

Results (Fig. 1)

Before immunotherapy

The median (minimum–maximum) SI of controls (2.39, 0.52–3.39) was compared to the median (minimum–maximum) of the SI in patients (10.13, 1.19–44.88) and was found to be significantly lower ($P = 0.006$). Thirteen of the 18 patients (72%) were positive ($SI > 3.39$). Baseline median venom-specific serum IgEs of patients were 7.37 kU/L (0.40–44.88).

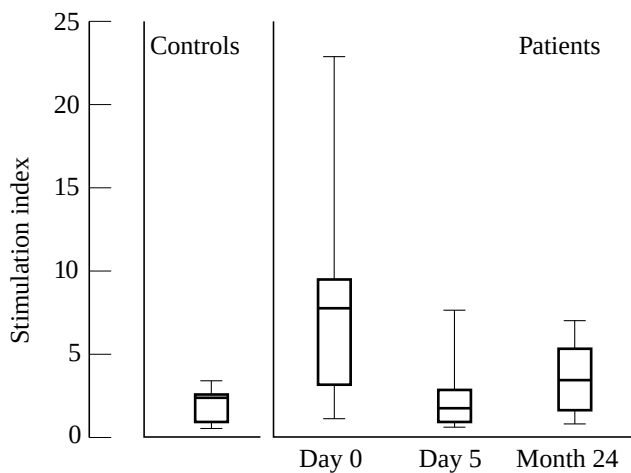


Fig. 1. Medians and whisker plots (25–75%ile) of the venom stimulations in controls and patients, at the different time points.

Evolution of the immune parameters during immunotherapy

At the end of the build-up immunotherapy

When LTT was repeated in the patients at the end of a 5-day semi-rush build-up hyposensitization course, SI remained positive in 3/18 (17%) of the patients. Nine of the 13 (69%) initially positive patients turned negative. The median (minimum–maximum) 2.73 (0.67–12.03) was significantly lower as compared to the baseline values ($P = 0.002$). In addition, the proliferative response to *Vespula* venom at d5 in patients was not significantly different from controls.

At 24 months of maintenance immunotherapy

At 24 months, the SI values were 2.39 (1.12–6.02) in controls and 4.21 (0.88–14.66) for patients respectively. The values for controls did not differ significantly between day 0 and month 24. On the contrary, although higher than at day 5, the results in patients were significantly lower at month 24 as compared with day 0 ($P = 0.03$) and medians did not differ significantly from controls ($P = 0.5$). At month 24, 10 of the 18 patients had still positive SI values, but the difference in frequency was not statistically significant (chi-square day 0, month 24: $P = 0.17$). At month 24 the mean *Vespula* specific IgE was 3.25 (0.17–16.00); 16 of the 18 patients had still positive values.

Discussion

Diagnosis of vespid anaphylaxis is regularly made by a positive history, backed up with a positive specific IgE. The primary objective of the present study was to measure

Vespula venom specific lymphoblastogenesis in vespid-allergic patients and in stung controls, in order to examine the hypothesis that the LTT to wasp venom might also be a discriminative parameter between vespid-allergic patients and stung controls. Eighteen patients, with a compelling history of a severe wasp sting induced anaphylaxis and with a positive serum venom-specific IgE and seven non-symptomatic stung control individuals with negative specific IgE were studied. The pre-treatment SI values in patients were significantly higher than in controls. Thirteen out of 18 patients had a positive LTT (sensitivity 72%). These results demonstrate that the LTT is a less sensitive marker than the determination of specific IgE for the diagnosis. Neither serum venom-specific IgE nor venom skin test reflect clinical insensitivity and are not of any predictive value at the end of the build-up course or early maintenance immunotherapy [12,15,16]. By sting challenge, protection can be demonstrated by a well-tolerated re-exposure after a semi-rush or rush hyposensitization. Unfortunately, a deliberate sting challenge remains expensive, is inconvenient for the patient and is not acceptable for a number of clinicians. In order to evaluate the possible value of the LTT in stopping immunotherapy, we first had to demonstrate that the results of the LTT paralleled clinical insensitivity. Therefore, LTT was repeated at the end of a 5 day build-up VIT, at a moment when it is well known from previous studies that patients become clinically insensitive to sting challenges [15]. When the SI values at d5 were compared with baseline (d0) values, a highly statistically significant decrease was observed. Of special interest was the observation that, probably as a consequence of the given amount of antigen, in a majority of patients (12/18) the LTT values became negative at the end of the build-up course of VIT. Since most of the patients are clinically insensitive to subsequent stings at the end of the build-up therapy [15], we speculated that the abolition of the LTT might be an *in vitro* correlate of clinical insensitivity. In contrast to our findings, Lesourd *et al.* [17,18], calculating T proliferative and T suppressor activity after an 8 day rush venom immunotherapy via venom allergen stimulation of lymphocytes, found increased values during immunotherapy. However, their stimulation method differed from the classical LTT stimulation.

The immunological parameters used most frequently to evaluate the response to VIT are the venom-specific IgE antibody levels and the venom skin test. Both venom specific IgE and venom skin test reactivity slowly decrease during prolonged VIT, becoming completely negative in only a minority of patients. Abolition of venom skin-test reactivity has been reported to occur in only 20–30% of the treated patients after 3–5 years of VIT [1,8,13]. We therefore also focused our attention on the evolution of the LTT during immunotherapy. Based on our findings of the

impressive decrease of the LTT values at day 5, we suggest that if the LTT turns negative after prolonged immunotherapy, this might indicate clinical protection. As a first step in evaluating this hypothesis, we therefore extended our study by repeating the LTT at 24 months of maintenance therapy. As presented in Fig. 1, the SI of the LTT remained decreased during VIT and was negative in 10 of 18 patients, at month 24. In contrast, negative wasp *Vespula*-specific IgE was only observed in two of 18 patients. These results are encouraging because they suggest that, in the majority of patients treated with VIT, a negative LTT might be a good *in vitro* correlate of clinical insensitivity. Moreover, LTT might probably contribute in the decision of stopping hyposensitization especially in patients who have a positive LTT before immunotherapy. The LTT offers no information for the patients negative at the start of the immunotherapy. This study will be continued until patients reach 5 years of immunotherapy. Further studies, relating LTT results during venom immunotherapy with natural stings will be necessary to confirm our hypothesis.

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