

Food, drug, insect sting allergy, and anaphylaxis

Venom immunotherapy reduces large local reactions to insect stings

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Background: Large local reactions to insect stings cause significant morbidity and impair quality of life. Venom immunotherapy is not recommended because of a low risk for future systemic reaction and unproven efficacy in preventing large local reactions.

Objective: To determine the feasibility of performing a controlled trial to examine the efficacy of venom immunotherapy in reducing the size and duration of large local reactions.

Methods: Sting challenge in 41 patients with previous large local reactions and positive venom skin tests caused large local reactions 16 cm or larger in 34 patients, and 29 consented to treatment. Venom immunotherapy was initiated in 19, and 10 were untreated controls. Sting challenge was repeated after 7 to 11 weeks (control patients then began venom immunotherapy), and annually for as long as 4 years.

Results: After 7 to 11 weeks of treatment, the size and duration of large local reactions decreased 42% and 53%, respectively, in treated patients and 18% in controls ($P < .01$ for both). The response was similar after 1 year, and improved after 2 to 4 years to 60% and 70%, respectively.

Conclusions: Venom immunotherapy significantly reduced the size and duration of the large local reactions, and the efficacy improved over a period of 2 to 4 years of treatment. Further studies are needed to establish the safety and efficacy of venom immunotherapy for large local reactions, the optimal duration of treatment, and the mechanism for the differences in degree and rate of clinical response compared with venom immunotherapy in systemic reactors. (J Allergy Clin Immunol 2009;123:1386-90.)

Key words: Insect sting, venom immunotherapy, large local reaction, *Hymenoptera*

Abbreviations used

LLR: Large local reaction

VIT: Venom immunotherapy

Large local reactions (LLRs) to *Hymenoptera* stings are IgE-dependent and are estimated to occur in 10% of adults.¹ They follow the pattern of late-phase inflammatory responses, with swelling delayed 6 to 12 hours in onset, peaking by 24 to 48 hours and resolving in 3 to 10 days. There is no established definition of the size of a LLR, but they are commonly 4 to 6 inches (10-15 cm) in diameter. They can result in massive swelling of an entire extremity, can cause significant morbidity and impairment in quality of life, and often require a course of oral corticosteroids.^{2,3}

Venom immunotherapy (VIT) is currently indicated only to prevent anaphylactic reactions to stings in patients with a history of previous systemic reactions and positive venom skin tests or serum venom-specific IgE serology.⁴ VIT is not considered cost-effective for patients with only LLR, because their risk of future sting anaphylaxis is relatively low. However, patients who have frequent and disabling LLR, particularly those with occupational exposure, have requested VIT. Although they desire treatment in the hope it would minimize a future LLR, the efficacy of such treatment is unproven. There is reason to hope that venom immunotherapy would mimic the natural reduction of severe LLRs that has been reported in patients who have multiple and frequent honeybee stings or mosquito bites.⁵⁻⁷

This pilot study was designed to evaluate the feasibility of a clinical trial of the efficacy of VIT in reducing the size and duration of LLR to *Hymenoptera* stings. Our specific aims were to determine the interest of affected patients in VIT, to compare the duration and peak size of the sting reaction after 7 to 11 weeks of either VIT or no treatment, to describe the change in duration and peak size of the sting reaction after 1 to 4 years of VIT, to observe adverse reactions to VIT in large local reactors, and to observe changes in venom skin tests and IgE antivenom levels in serum during the study period.

METHODS

We recruited subjects from groups of previous study volunteers as well as from the community by advertising. Individuals were included with a convincing history of a large local sting reaction and positive venom skin test results. Exclusions included the use of β -blocker medication, pregnancy or insufficient measures to avoid pregnancy, previous sting anaphylaxis or venom immunotherapy, and uncontrolled asthma or other medical conditions that might affect the risk or outcome of anaphylaxis. Subjects were enrolled

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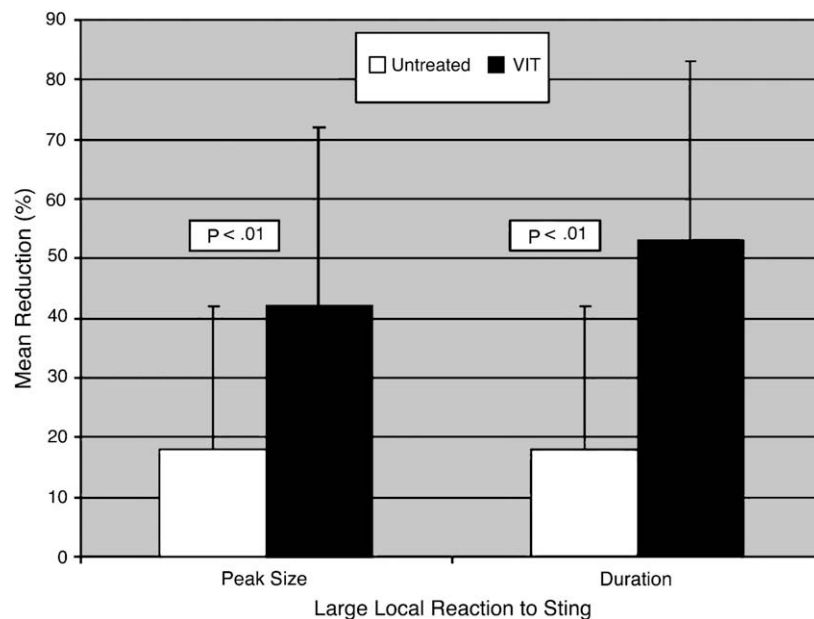


FIG 1. Mean reduction $\pm$ SD (%) in the peak size and duration of large local reactions to stings after 9 weeks (range, 7-11) of either initial VIT (19 subjects) or no treatment (10 subjects). There was a significantly greater improvement in the treated subjects.

over a 3-year period, with informed consent about the benefits, risks, and alternatives, including medical treatment. The study protocol and consent form were approved by the Johns Hopkins Investigation Review Board.

To establish a suitable baseline before treatment, a qualifying sting challenge was performed. Only subjects who developed local induration of at least 16 cm were eligible to continue in the treatment phase of the study. This threshold for inclusion was chosen to facilitate the detection of a therapeutic effect, and because such patients are more motivated to undergo VIT. Venom immunotherapy was administered according to all our usual clinic protocols, using venoms donated by ALK-Abelló Laboratories, Round Rock, Tex, with a dose schedule adapted from the package insert to achieve the maintenance dose in 7 weekly visits. Maintenance doses (100 μ g of each venom; 300 μ g for mixed vespoid venom) were administered every 4 weeks for 1 year, then every 6 weeks for 1 year, and then every 8 weeks for 2 years. Patients were encouraged (but not required) to take an antihistamine dose the morning of each treatment day (cetirizine or fexofenadine), but not for 1 week before the sting challenge. Untreated patients in the control group did not take either active antihistamine or placebo medication during the same period, but neither group took antihistamines for 1 week before repeat sting challenge.

Sting challenges were performed as previously described,⁸ with institutional review board–approved informed consent at Johns Hopkins Bayview Medical Center and at Penn State Hershey Medical Center. We received invaluable assistance from the facility and staff of the National Institutes of Health–funded General Clinical Research Centers at both locations. Before the sting, venom skin tests were performed, an intravenous infusion was established, and blood samples were drawn. Patients were closely monitored for symptoms and objective signs of systemic reaction for at least 60 minutes after the sting. No additional medications were permitted for the first 48 hours after the sting to enable accurate measurement of the cross-sectional diameters of the swelling. At the time of qualifying sting challenge, patients were trained in the measurement and recording of the maximal cross-sectional diameters of the area of local induration resulting from the sting. The size of the local reaction (centimeters of induration) was recorded by the patient at 1, 12, 24, and 48 hours after the sting. After the 48-hour period of observation, subjects were permitted to take antihistamines and/or a short burst of prednisone.

Venom skin tests were performed by using a standard intradermal technique involving venom concentrations from 0.001 to 1.0 μ g/mL. This titration was performed to determine the lowest concentration that would

produce a positive result (wheal >5 mm and erythema >10 mm). Venom-specific IgE antibodies were measured in serum samples by using the Pharmacia ImmunoCAP-250 system (Pharmacia, Uppsala, Sweden). Sting challenge was performed by using a device and procedure we have previously described.⁸ The insect that was implicated by the patient's history and venom skin test results was used for sting challenge. The use of yellow jacket stings in patients with multiple vespoid venom sensitivity and treatment was based on the patient's history. The sting application devices and the insects were provided by our collaborating entomologists at the University of Maryland (Nancy Breisch, PhD and Al Greene, PhD).

Comparison of outcomes between VIT and untreated groups was performed by using the Student *t* test in SigmaStat 9.0 (Systat Software Inc, Point Richmond, Calif).

RESULTS

Of the 200 who responded to advertisements, relatively few were interested in proceeding with treatment. The patients' interest in treatment was related not only to the severity of their reaction but rather to the frequency of their reactions and their frequent exposure to stinging insects. Although many subjects chose medical management over the study treatment, the study subjects expressed a motivation to avoid not only the frequent sting reactions but also the need for repeated courses of steroids.

An initial (qualifying) sting challenge was performed in 41 subjects: 17 men and 14 women, mean age ($\pm$ SD) 46.5 ($\pm$ 11) years (range, 21-63). During the initial sting challenge, 1 subject (2.4%) had an anaphylactic reaction, which required epinephrine and intravenous fluids for an extended period. There were no other systemic reactions, but 6 subjects had local reactions that were not large enough to qualify for the treatment phase of the study. Of the 34 patients who qualified, 5 declined treatment. A total of 29 patients were treated with venom immunotherapy: 7 with honeybee venom, 2 with yellow jacket venom, 18 with mixed vespoid venom, and 2 with *Polistes* wasp venom. In these 29 subjects, venom skin tests were positive at 0.01 μ g/mL in 3 cases, at 0.1 μ g/mL in 11, and at 1.0 μ g/mL in 15 patients.

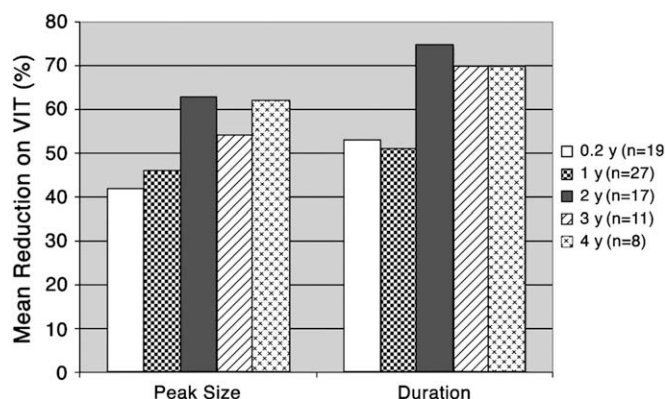


FIG 2. Mean reduction (%) in the peak size and duration of large local reactions to stings after initial VIT up dosing (0.2 years; $n = 19$) and after maintenance VIT for 1 year ($n = 27$), 2 years ($n = 17$), 3 years ($n = 11$), and 4 years ($n = 8$). Improvement was greater after 2 to 4 years than after the first year of treatment.

The controlled phase of the study was the first 12 weeks after the first (ie, qualifying) sting challenge, in which the patients were assigned in a 2:1 ratio to VIT ($n = 19$) or no treatment ($n = 10$). Sting challenge was repeated after 7 to 11 weeks. The untreated patients were then offered VIT, and 9 accepted. Sting challenge was repeated annually for 1 to 4 years. VIT was well tolerated by all study subjects. There were no systemic reactions to injections. LLRs to injections were no more common than normally observed in treatment of patients with previous systemic sting reactions. There were 8 subjects who complained of a large area of swelling after an injection: 1 took prednisone for 2 days on 1 occasion, 5 took only antihistamine, and 2 took no additional medication. Adverse effects did not lead to any delays in the treatment schedule or withdrawals from the study.

The mean reduction, from the first (qualifying) sting to the second sting, in the peak size and duration of the LLR in untreated and VIT patients is depicted in Fig 1. Untreated patients showed a mean ($\pm$ SD) decrease of 18% in both their peak size ($\pm 21\%$) and duration of reaction ($\pm 26\%$). Patients who received VIT had a mean ($\pm$ SD) reduction of 42% ($\pm 30\%$) in the peak size, and 53% ($\pm 31\%$) in the duration of the reaction. This was a significantly greater improvement than in untreated patients for both the size ($P = .008$) and duration ($P = .01$) of the post-sting reaction. The reduction (from the qualifying sting) in size and duration of the LLR to sting challenge after 1 to 4 years of VIT is shown in Fig 2. The improvement after 1 year was not different from that observed after the first 7 to 11 weeks. The results improved after 2 years of treatment and were maintained through 4 years of VIT, with about a 60% reduction of the size, and a 70% reduction in duration of the swelling.

A clinical response to VIT was defined as at least a 50% improvement in size or duration of the sting reaction. Fig 3 displays the response rates over the course of the study. After 7 to 11 weeks, the response rates were 37% and 53% for size and duration, respectively, compared with 10% for untreated controls. The response rate increased to 48% and 63%, respectively, after 1 year of VIT, 76% and 94% after 2 years, 73% and 82% after 3 years, and 87% for both after 4 years. Patients expressed satisfaction with treatment when there was at least 50% reduction in either the size or duration of their reaction. Patient satisfaction

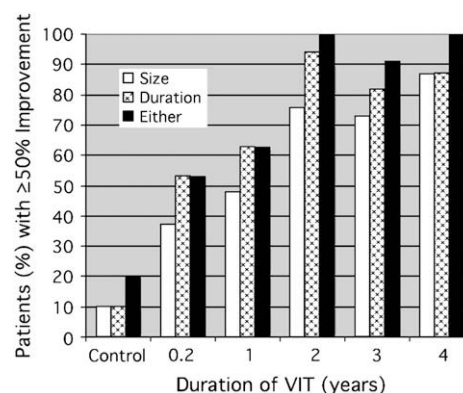


FIG 3. Clinical response rate (percentage of patients with at least 50% improvement in size or duration of the sting reaction) is shown after initial VIT up dosing (0.2 years; $n = 19$), and after maintenance VIT for 1 year ($n = 27$), 2 years ($n = 17$), 3 years ($n = 11$), and 4 years ($n = 8$), as well as in untreated controls.

was 53% after 7 to 11 weeks of VIT, 63% after 1 year, 100% after 2 years, 91% after 3 years, and 100% after 4 years.

The changes observed in the subjects' venom skin tests over the 4-year period of the study are presented in Table I. After the first year of treatment, there was little overall change in skin test results, with 15 patients showing the same endpoint titer as before treatment, 8 showing increased skin sensitivity, and 5 showing reduced sensitivity. Over time, an increasing number of patients showed reduced skin reactivity: 18% of the subjects after 1 year, 41% after 2 years, 64% after 3 years, and 75% after 4 years of VIT. Skin test results became negative in 18% of patients after 1 year, 24% after 2 years, 43% after 3 years, and 38% after 4 years of VIT. Venom-specific IgE antibody levels (data not shown) increased during the initial treatment phase (after qualifying sting challenge), particularly in the treated subjects. IgE antivenom levels in serum were still elevated at 1 year, and then returned to baseline levels in years 2 to 4. The venom-specific IgE antibody level did not convert to negative (<0.35 kU/L) in any patient over the course of the study.

DISCUSSION

Venom immunotherapy has proven to be effective for the prevention of sting anaphylaxis.⁹⁻¹¹ However, there have been few reports in the literature on the prevention of morbidity associated with LLRs using immunotherapy. An abstract by Walker et al¹² showed that rush immunotherapy prevented LLRs to fire ant stings. Schuberth et al¹³ reported that significantly fewer LLRs occurred in venom-treated (compared with untreated) children who had a history of previous mild systemic reactions to stings. Most recently, the initial response to 6 months of sublingual immunotherapy with honeybee venom was reported to be associated with reduced LLRs to stings and increased venom-specific IgG antibody levels.¹⁴ We reported a single case of a patient who had marked reduction of LLRs with VIT.¹⁵ Immunotherapy has been shown to attenuate the late-phase cutaneous inflammatory reaction to venom and inhalant allergens.¹⁶⁻¹⁸ Our data convincingly demonstrate the clinical efficacy of VIT in attenuating the late inflammatory allergic response to a sting. This finding is expected on the basis of similar observations in patients with LLRs to honeybee stings or mosquito bites who showed diminished reactions after a large number of natural stings or bites over time.^{5,7,19}

TABLE I. Changes in skin test sensitivity during venom immunotherapy: numbers of patients showing stronger, weaker, or unchanged skin test responses

Duration of VIT	No.	Change in skin test after first sting, n (%)		
		Same	Stronger	Weaker/negative
1 y	28	15 (54)	8 (29)	5 (18)/5 (18)
2 y	17	8 (47)	2 (12)	7 (41)/4 (24)
3 y	14	4 (29)	1 (7)	9 (64)/6 (43)
4 y	8	2 (25)	0 (0)	6 (75)/3 (38)

The patients were advised of the alternatives for medical treatment instead of VIT, particularly a short course of oral corticosteroids. Although most experts believe this treatment to be quite effective, it is best initiated as soon as possible after the sting.⁴ There is no published evidence to support this contention, and no controlled trial has been published. It is therefore very difficult to compare the benefits of VIT and medical management. However, VIT would appear to be a steroid-sparing treatment option in these patients who are motivated to avoid not only the frequent sting reactions but also the need for repeated courses of steroids.

The time course and pattern of the clinical response to VIT observed in the current study of large local reactors were somewhat different than has been observed in systemic reactors. As with systemic reactions, there was evidence of efficacy for LLRs even in the early stage of maintenance therapy. However, optimal clinical benefit for LLRs did not occur in the first season of treatment, as has been noted in systemic reactors. Rather, it was observed in the third sting season, after 2 full years of treatment. The frequency and degree of the clinical response to VIT also appeared different between the large local and the systemic reactors. VIT in systemic reactors completely prevents the systemic manifestations, but in large local reactors, the improvement appears to be less than complete. The frequency of clinical response (no systemic reaction to a sting) to VIT in systemic reactors is 85% to 98%, but the current study shows that the response rate (as well as the mean reduction in LLR) was only 50% to 60% in the first 2 seasons of treatment. The initial response rate was similarly low in the reported study of sublingual VIT for LLRs, in which the treatment was studied for only 6 months.¹⁴ In published comments on that study, the response rate was judged to be inferior to that of VIT for systemic reactions, and it was noted that in studies of systemic reactors, even the placebo response rate approached 50%.²⁰ In the current study, only 10% of the untreated patients had at least a 50% improvement, and the mean reduction was 18%. The initial response rate in treated subjects in our study was indeed only about 50%, but it was significantly better than in the untreated group. More importantly, we demonstrated an improved response rate of more than 80% (with 60% to 70% reduction in size and duration of reaction) after 2 to 4 years of VIT. In the current study, there was another potential difference between the treated and control patients: only treated patients used an antihistamine weekly for the first 7 weeks. Neither group used an antihistamine for 1 week before challenge sting, but we cannot assess any potential effect of the antihistamine treatment on the clinical outcome. There have been recent reports suggesting possible mechanisms for immunomodulatory effects of antihistamines during VIT.²¹

The improved response after 2 to 4 years of VIT in the current study is reported in a smaller number of subjects than the results

after 1 year. This was not a result of the nonresponders dropping out of the study. There were 10 patients (5 controls and 5 VIT) who joined the study in its penultimate year, and so they had results available for only 1 year. These patients had results comparable to those of the other 19 subjects who joined the study during its first 2 years. All but 1 of the subjects who showed a poor response in the first year showed an improved response in years 2 to 4.

Although the observed difference between the treated and control groups was statistically significant after the initial venom treatment, there were shortcomings in the design of this pilot study. The control group received no treatment rather than a placebo treatment. Such unblinded, non-placebo-controlled comparisons are potentially susceptible to bias, although the outcomes were strictly objective. Furthermore, the control group was crossed over to VIT treatment after the first 3 months to increase the number of treated patients who could be evaluated in this pilot design. Therefore, there was no control group for comparison during the extended maintenance period of as long as 4 years. It is not possible to know whether reduced reaction to stings could have occurred spontaneously over the period of 2 to 4 years. There have been few studies of the natural history of LLRs, but published reports describe continued severe reactions in many cases.^{2,3,22} This is also reflected in the observation that of the 41 subjects with previous LLRs who underwent an initial sting challenge in this study, all developed LLRs, and 34 of 41 (83%) were 16 cm in diameter or larger. Our results support the need for a more rigorous study involving a larger number of patients to confirm the observed efficacy and to determine the most appropriate timing and duration for maintenance treatment.

This study was not designed to make detailed immunologic observations; however, changes were observed in venom skin test endpoint titer and IgE antivenom levels in serum during the study period. Venom skin tests showed unexplained variability in individual patients, but they showed progressively declining sensitivity during the 4-year treatment period. The variability of venom skin tests has been previously reported.²³ The lack of a control group during maintenance VIT makes it uncertain how much of the decline in sensitivity was a result of treatment and how much was simply the result of the passage of time. As observed in previous studies of VIT, venom-specific IgE increased with initial VIT and then declined toward baseline levels after 1 to 2 years of treatment.²⁴ Also as previously reported for VIT in systemic reactors, serum venom-specific IgE antibodies remained detectable in patients whose venom skin test results became negative during VIT. We believe this to be related to the limited sensitivity of venom skin tests^{25,26} rather than potential differences in the commercial venom products used for skin tests and serologic methods.

The mechanism by which VIT induces a reduction in the LLR may be more complex than in prevention of anaphylaxis. The limited response in the first year suggests that there may be both

IgE-mediated mechanisms that do respond to early treatment and mechanisms that are non-IgE mediated (or are not responsive to VIT). The improved response after 2 to 4 years suggests the possibility of a suppressive mechanism that becomes active only after prolonged treatment, like the long-lasting protection (tolerance) that results from 4 to 5 years of VIT in systemic reactors (which is independent of IgG antibodies). Venom-specific IgG antibodies have been implicated in the diminishing LLRs that occur with very frequent exposure in beekeepers and individuals with mosquito allergy.⁵⁻⁷ Further study of VIT for LLRs may be a useful model to elucidate the mechanisms of both LLRs and of VIT, especially by comparison with the responses in systemic reactors.

Our clinical observations support the safety of VIT in patients with a history of LLRs. There were no significant adverse events during treatment. Surprisingly, LLRs to venom injections occurred no more often than during VIT in systemic reactors. One interesting observation was the outcome of the pretreatment qualifying sting challenge. Almost all the subjects had reactions reproducing their previous reactions. This suggests that these patients were likely to continue to have similar LLRs to future stings. As predicted, there was a systemic reaction to the initial sting challenge in less than 5% of the subjects. Of interest is the anecdotal observation that when the initial systemic reaction occurs, it can be of any severity, including life-threatening, as was the case in this study.

One aim of this pilot study was to evaluate the feasibility of recruiting patients for a large study. Only a small number of patients with LLRs were interested in treatment, particularly those who had episodes almost every summer in which they experienced significant morbidity and often required medical attention and oral or injected corticosteroid treatment. The majority of patients with LLRs to stings have infrequent reactions and may be counseled to begin a brief course of prednisone as soon as possible after the sting. To enroll sufficient patients, a well designed large-scale study would therefore have to be performed in multiple study centers.

The results of the current study provide preliminary data to support several conclusions. VIT significantly reduces the size and duration of LLRs to stings. The clinical improvement in LLRs observed with VIT improves further during 2 to 4 years of treatment. Patient satisfaction with VIT was over 90%. Additional studies are needed to confirm the efficacy of VIT for prevention of LLRs and to determine the optimal duration of treatment. These studies will also present a unique opportunity to investigate the pathophysiology of large local allergic reactions and the mechanism by which immunotherapy inhibits this response.

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Clinical implications: Venom immunotherapy prevents severe large local reactions to *Hymenoptera* stings and may be considered in patients who experience frequent and severe sting reactions.

REFERENCES

1. Golden DBK, Marsh DG, Kagey-Sobotka A, Addison BI, Freidhoff L, Szklo M, et al. Epidemiology of insect venom sensitivity. *JAMA* 1989;262:240-4.
2. Green A, Reisman R, Arbesman C. Clinical and immunologic studies of patients with large local reactions following insect stings. *J Allergy Clin Immunol* 1980; 66:186-9.
3. Mauriello PM, Barde SH, Georgitis JW, Reisman RE. Natural history of large local reactions from stinging insects. *J Allergy Clin Immunol* 1984;74:494-8.
4. Moffitt JE, Golden DBK, Reisman RE, Lee R, Nicklas R, Freeman T, et al. Stinging insect hypersensitivity: a practice parameter update. *J Allergy Clin Immunol* 2004;114:869-86.
5. Bousquet J, Menardo J-L, Aznar R, Robinet-Levy M, Michel F-B. Clinical and immunologic survey in beekeepers in relation to their sensitization. *J Allergy Clin Immunol* 1984;73:332-40.
6. Light W, Reisman RE, Wypych JJ, Arbesman CE. Clinical and immunologic studies of beekeepers. *Clin Allergy* 1975;5:389-95.
7. Peng Z, Ho MK, Li C, Simons FE. Evidence for natural desensitization to mosquito salivary allergens: mosquito saliva specific IgE and IgG levels in children. *Ann Allergy Asthma Immunol* 2004;93:553-6.
8. Golden DBK, Breisch NL, Hamilton RG, Guralnick MW, Greene A, Craig TO, et al. Clinical and entomological factors influence the outcome of sting challenge studies. *J Allergy Clin Immunol* 2006;117:670-5.
9. Brown SG, Wiese MD, Blackman KE, Heddle RJ. Ant venom immunotherapy: a double-blind placebo-controlled crossover trial. *Lancet* 2003;361:1001-6.
10. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978; 299:157-61.
11. Muller U, Thurnheer U, Patrizzi R, Spiess J, Hoigne R. Immunotherapy in bee sting hypersensitivity: bee venom versus wholebody extract. *Allergy* 1979;34:369-78.
12. Walker R, Jacobs J, Tankersly M, Hagan L, Freeman T. Rush immunotherapy for the prevention of large local reactions secondary to imported fire ant stings. *J Allergy Clin Immunol* 1999;103:S180.
13. Schuberth KC, Kagey-Sobotka A, Kwitrovich K, Graft DF, Szklo M, Lichtenstein LM, et al. A comparison of venom treatment and no treatment in insect allergic children [abstract]. *J Allergy Clin Immunol* 1982;69:124.
14. Severino MG, Cortellini G, Bonadonna P, Francescato E, Panzini I, Macchia D, et al. Sublingual immunotherapy for large local reactions caused by honeybee sting: a double-blind placebo-controlled trial. *J Allergy Clin Immunol* 2008;122:44-8.
15. Hamilton RG, Golden DBK, Kagey-Sobotka A, Lichtenstein LM. Case report of venom immunotherapy for a patient with large local reactions. *Ann Allergy Asthma Immunol* 2001;87:134-7.
16. Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997;99:254-60.
17. Nasser SMS, Ewan PW. Effect of wasp venom immunotherapy on the venom-induced cutaneous late-phase reaction. *Allergy* 1997;52(suppl 37):88 [abstract].
18. Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993;92: 644-51.
19. Light WC, Reisman RE, Shimizu M, Arbesman CE. Unusual reactions following insect stings. *J Allergy Clin Immunol* 1977;59:391-7.
20. Rueff F, Bilo B, Jutel M, Mosbech H, Muller U, Przybilla B. Sublingual immunotherapy with venom is not recommended for patients with Hymenoptera venom allergy [letter]. *J Allergy Clin Immunol* 2009;123:272-3.
21. Muller UR, Jutel M, Reimers A, Zumkehr J, Huber C, Krieger C, et al. Clinical and immunologic effects of H1 antihistamine preventive medication during honeybee venom immunotherapy. *J Allergy Clin Immunol* 2008;122:1001-7.
22. Graft DF, Schuberth KC, Kagey-Sobotka A, Kwitrovich KA, Niv Y, Lichtenstein LM, et al. A Prospective study of the natural history of large local reactions following Hymenoptera stings in children. *J Pediatr* 1984;104:664-8.
23. Graif Y, Confino-Cohen R, Goldberg A. Reproducibility of skin testing and serum venom-specific IgE in Hymenoptera venom allergy. *Ann Allergy* 2006;96:24-9.
24. Golden DBK. Stinging insect vaccines: patient selection and administration of Hymenoptera venom immunotherapy. In: Lefford DK, Lockey RF, editors. *Immunotherapy: a practical review and guide*. Philadelphia: W.B. Saunders; 2000. p. 553-70.
25. Golden DBK, Kagey-Sobotka A, Hamilton RG, Norman PS, Lichtenstein LM. Insect allergy with negative venom skin tests. *J Allergy Clin Immunol* 2001;107: 897-901.
26. Golden DBK, Kelly D, Hamilton RG, Wang NY, Kagey-Sobotka A. Dialyzed venom skin tests for identifying yellow jacket-allergic patients not detected using standard venom. *Ann Allergy Asthma Immunol* 2009;102:7-50.