

Hymenoptera Venom Extracts in Clinical Practice

James M. Tracy, DO^a, and David B. K. Golden, MD^b Omaha, Neb; and Baltimore, Md

Venom immunotherapy is the only treatment for the prevention of anaphylaxis and remains the prototypical model for its diagnosis and management. Unlike other causes of anaphylaxis, such as foods and drugs, preventative treatment using venom immunotherapy is highly effective with well-established protocols, and is widely available. Hymenoptera venoms are also unique in that the primary allergens are directly measurable in terms of micrograms, rather than labeled on the basis of potency. As such, venoms were the first truly standardized extracts. However, Hymenoptera venom presents unique challenges in collection, processing, and stabilization of the venom extract product. Historically, there have been minor variations in insect composition in venom extract content. These differences were in part based on variation in insect availability and venom collection and have created minor challenges. However, it is a recognized need that venom extracts contain an optimal and complete repertoire of relevant venoms so as to be assured of appropriate diagnosis and treatment. As such, insect availability and collection, as well as storage, stability, and sterility of venom extracts, have remained critical components to appropriate diagnosis and prevention of venom-induced anaphylaxis. In recent years there has been concerns about adequate supplies of venom for appropriate diagnosis and treatment of venom allergy, but these concerns seem to have stabilized, with the movement to a single venom supplier. This movement to a single supplier may provide its own challenges. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1856-62)

Key words: *Venom immunotherapy; Hymenoptera; Allergy extracts; Venom skin testing; Insect allergy*

BACKGROUND

Among the allergenic extracts in clinical use, Hymenoptera venoms (HVs) are unique in many ways. First, the primary allergens are measurable. Aeroallergen extracts are marketed with labeled potency represented as various defined units such as

weight to volume (w/v), allergy units, or bioequivalent allergy units (all of which are American), or standardized quality (SQ) units, index of reactivity (IR) units, and others (in Europe). Venom extracts are the only allergen extracts whose potency is measured in micrograms of allergen per milliliter and as such they are truly standardized. Venoms were the first standardized allergens introduced into clinical practice, and one of the only truly new allergen extracts to become available in the past 40 years (dust mite extracts followed shortly after venom). They remain the only treatment approved for the prevention of anaphylaxis. The story of how pure, eventually commercially available, venoms came to replace whole-body extracts (WBEs) is a lesson in the value of studying epidemiology, observing the natural history, and performing controlled clinical trials.^{1,2}

The development of HV extracts presented unique challenges in collection, processing, and stabilization of the venom product. Clinical validation was also fraught with new problems in that the outcome measure was a life-threatening reaction. Nevertheless, thanks to the early efforts of Mary Loveless, Al Benton, Larry Lichtenstein, and his team at Johns Hopkins, venom extracts became a model for the study and treatment of anaphylaxis and immunotherapy.³⁻⁶

The development of HV extracts for clinical use had taken a wrong turn in 1930, when Benson and Semenov⁷ came to the conclusion that the relevant allergen was present equally in the body and venom of the insect. Loveless and Fackler⁶ reported that venom was the most effective insect component for immunotherapy, but their reports were anecdotal. Unfortunately, large surveys led to the endorsement of WBE by the Insect Committee of the American Academy of Allergy in 1965.⁸ It was not until 1976 that it was shown in a randomized controlled clinical trial that WBE was no better than placebo and that venom immunotherapy (VIT) gave 98% protection from anaphylaxis.⁴

CASE PRESENTATION

Part 1: Clinical history

While gardening, a 26-year-old gravida 1, para 0, female at 26 weeks estimated gestational age was stung by a ground-dwelling bee. Within 3 to 5 minutes she experienced a general feeling of anxiety, throat fullness, and some mild hives. After taking diphenhydramine and not improving she went to the emergency room where she was given additional diphenhydramine and observed for a period of 90 minutes. She continued to do well and was discharged with no follow-up or other specific treatment. After completing her pregnancy without further incident, she was once again stung approximately a year later, while camping with family. She was taken to the emergency room, and because of generalized hives and shortness of breath she was treated with epinephrine and admitted for observation. She was prescribed an epinephrine autoinjector and referred to an allergist-immunologist for further evaluation and treatment.

^aAllergy, Asthma and Immunology Associates, P.C., Omaha, Neb

^bDepartment of Medicine, Johns Hopkins University, Baltimore, Md

Conflicts of interest: J. M. Tracy declares receiving honorarium from UpToDate. D.

B. K. Golden declares Speaker Bureau honoraria from Genentech and Stallergenes; research support for clinical trials from Genentech and Stallergenes; and receiving royalties as an Editor for UpToDate.

Received for publication May 25, 2018; revised August 7, 2018; accepted for publication August 8, 2018.

Available online September 1, 2018.

Corresponding author: James M. Tracy, DO, Allergy, Asthma and Immunology Associates, PC, 2808 South 80 Ave, Ste 210, Omaha, NE 68124. E-mail: jtracy@allergynebraska.com.

2213-2198

© 2018 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaip.2018.08.015>

Abbreviations used

FDA- Food and Drug Administration

HB- honeybee

HV- Hymenoptera venom

VIT- venom immunotherapy

WBE- whole-body extract

YJ- yellow jacket

Clinical patterns of reaction and natural history.

Allergic reactions to insect stings can be local or systemic in distribution. Local reactions are usually not severe or persistent, but allergic swelling can be very large (16 cm or larger), delayed (peaking at 24-48 hours), and prolonged, subsiding in 5 to 10 days. These reactions can cause lymphangitic streaks toward inguinal or axillary lymph nodes that are often mistaken for a sign of cellulitis. Extreme swelling can involve an entire limb, and can cause a compartment syndrome or compression injury. Large local reactions are caused by a late-phase inflammatory allergic reaction to venom allergens. Any region of local inflammation, regardless of size, as long as it is contiguous with the sting site should be considered a local reaction and managed as such. Although most such patients may have venom-specific IgE no different from patients with anaphylactic reactions, they have a relatively benign natural history, with less than 10% chance of a systemic reaction to a future sting, and less than 3% chance of severe anaphylaxis.⁹ In comparison, epidemiologic observations in US adults showed about 1% will have a systemic reaction to a future sting and less than 0.5% will have anaphylaxis.^{10,11}

Systemic allergic reactions are generally immediate, beginning in minutes and causing any one or more of the symptoms and signs of anaphylaxis. Reactions limited to the skin (cutaneous systemic reactions) with urticaria, angioedema, flushing, or itching are considered mild and carry less than 3% risk of anaphylaxis to a future sting. Patients who have had moderate to severe anaphylactic reactions have a higher chance (30%-70%) of severe reaction to future stings. The risk is related to a number of factors, mostly the pattern and severity of previous sting reactions, the level of baseline serum tryptase, the level of platelet-activating factor acetylhydrolase, age, and concurrent medications.¹²

Types of insects and their venoms. Anaphylaxis to insects occurs with the stinging Hymenoptera (see Figure 1), but almost never with biting insects. The order Hymenoptera includes the bees, vespids (yellow jackets [YJs], hornets, and paper wasps), and stinging ants. Common terminology varies, and the term “wasp” is often used to describe all the vespid insects, and “bee” sometimes refers to any stinging insect. Although there are many species of Hymenoptera capable of inducing anaphylaxis, commercially available extracts exist for only a small proportion of these. Although there is substantial cross-reactivity among venoms of closely related species of Hymenoptera, some species do have unique antigens that do not cross-react with other members of the genus. Among the YJs and wasps, there are many species that are virtually identical allergenically, but *Vespula squamosa* (Southern YJ) and *Polistes dominula* (Mediterranean wasp) show unique antigenicity in comparison to other YJ and wasp venoms (respectively) that have been studied.

Venoms of the Dolichovespula (American hornets) and Vespa (Old World hornets) genera are less problematic with a relatively small number of closely related species. However, there remain significant differences in venom characteristics. Of the 5 species in North America, only 2 species are commonly encountered and linked to hypersensitivity reactions: *D armenaria* (yellow hornet) and *D maculate* (bald-faced, or white-faced hornet).¹³ There are commercially available extracts for each of these 2 individual species.

Compared with other flying Hymenoptera, honeybees (HBs) have unique venom allergens that show limited cross-reactivity with the other HVs. Unfortunately, there is incomplete cross-reactivity between HB venom and the 54 other native species of Apid bees encountered in North America, such as *Bombus terrestris*, the bumblebee. There are no commercially available extracts for any of the Apid bees other than HBs.

The stinging ants differ completely in their venom chemistry and their allergens, showing no significant cross-reactivity between their venoms and the other HVs, or between their own genera (Solenopsis, Myrmecia, Pogonomyrmex, Pachycondyla). The venom allergens are proteins, almost all of which have enzymatic activity (eg, phospholipases and hyaluronidases).^{14,15} Imported fire ant WBEs are available for *S richterii* and *S invicta*.

In each case, the venoms have a number of component allergens, but a single major allergen: Api m 1 in HBs, Ves v 5 and its homologues in the Vespula and Polistes venoms, and Sol i 1 in the imported fire ant venom. In a large minority of patients, there is also clinically significant sensitivity to 1 or more of the other component allergens in these venoms, so any venom extract should ideally contain all the relevant component allergens. A missing or inactive component could lead to a missed diagnosis or treatment failure. Recombinant venom allergens may therefore be very useful to distinguish cross-reactivity from dual sensitization (but may require the use of multiple component allergens for each venom). They may be of little use for optimal diagnostic accuracy unless all the components are present and fully active. In this case, like so many others, the culprit insect is unknown and testing to all potential insects from her region should be done.

Part 2: Venom testing

After a comprehensive history was taken and emergency room records were reviewed, no specific insect could be identified. A baseline serum tryptase and skin testing for all 5 available venoms were done. Serum tryptase was determined to be 6.2 ng/mL (normal, <11.4ng/mL) and skin testing using both puncture and intradermal methods was completed. Skin testing result for HB and white-faced hornet venoms was negative. However, testing results for YJ, yellow hornet, and wasp venoms were positive at 0.1 and 0.01 µg/mL, respectively. Because the culprit insect was not identifiable and the skin test results were not all positive, additional *in vitro* testing was completed using ImmunoCAP methods. The results showed an absence of HB, wasp, or white-faced hornet-specific IgE. However, testing results for YJ and yellow hornet were positive at 1.8 and 2.1 µg/mL, respectively (negative, <0.35 µg/mL).

Clinical utilization of venom extracts

Skin test/lab (specific serum IgE). In clinical use, the properties and characteristics of the venom extract products are

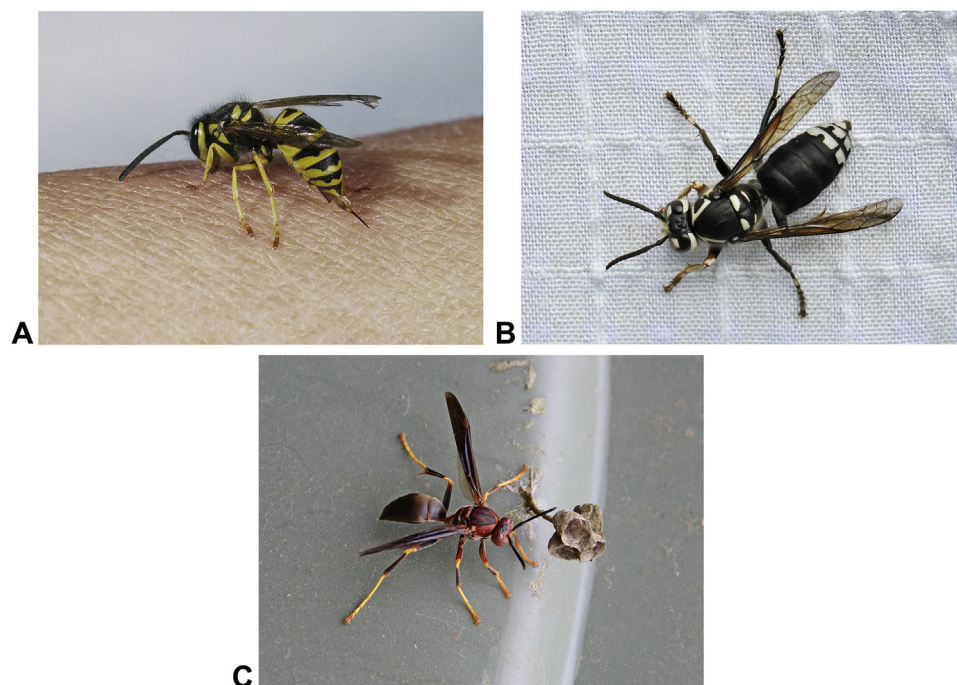


FIGURE 1. Examples of common stinging Vespid insects of North America. **A**, *Vespula maculifrons* worker (YJ). **B**, *Dolichovespula maculata* worker (bald-faced hornet). **C**, *Polistes metricus* queen (paper wasp). Reprinted with permission from Nancy Bresich, PhD, and Al Greene, PhD.

closely tied to the benefits, risks, doses, and regimens of VIT, and to the diagnostic protocols and accuracy for allergy testing.

Venom extracts are used for diagnostic skin testing. For intradermal tests, concentrations between 0.001 $\mu\text{g/mL}$ and 1.0 $\mu\text{g/mL}$ are typically used. The diagnostic accuracy of the test is potentially affected by whether all the relevant allergens are proportionately represented in the commercial venom extract. The presence of irritant components can cause false-positive reactions, and the stability of the venom at low concentrations could affect the reliability of the skin test results. Dialyzed (purified) venom extracts have been shown to provide greater diagnostic accuracy in skin testing, and are widely used in Europe because they have been shown to cause fewer adverse effects in VIT.^{16,17}

Venom extracts are also used for *in vitro* diagnostic testing. For this purpose, they must be dialyzed to remove beta-alanine and other factors that cause confounding background noise in the assay. The diagnostic accuracy of the ImmunoCAP assay for YJ venom-specific IgE has been improved by “spiking” the test with additional Ves v 5 allergen,¹⁸ and some patients might have false-negative test results for HB venom due to a relative insufficiency of Api m 10 in the reagent.¹⁹ Other *in vitro* diagnostic testing methods, not available in the United States, such as basophil activation as well as component-resolved diagnostic testing, may have clinical utility. Component-resolved testing in particular has been useful in diagnostic specificity, but has not added to diagnostic sensitivity.

It is important to note that the level of venom-specific IgE antibodies, in and of itself, is an imperfect predictor of a clinical reaction to a sting. The strength of the test result correlates with

the chance of reaction, but not as well with the severity of reaction. In fact, even among patients with previous sting anaphylaxis and clearly positive venom skin test results, only 60% reacted to live sting challenge.⁴ In the United States, live sting challenges are generally limited to the research setting and therefore not used in the typical practice setting. Nevertheless, sting challenge remains the “criterion standard” test of clinical reactivity.

Insect and venom collection. In the case of HBs, the venom can be collected by having them sting through a membrane against an electric grid that does not harm the bees or the venom.⁵ The dried venom collected from the membrane is then stored for later production. In the case of the vespids, the insects must be collected in a way that preserves the uniformity of the collected insects (species specificity) and the immunochemical integrity of the venom. A network of trained expert collectors will identify the insects, immobilize them with CO_2 , and keep them frozen until they are delivered to a central laboratory. The method of dissection and extraction of vespid venoms was described initially in the 50s, and developed further in the 70s to be used for commercial extract production.^{6,20} The venom sacs are removed from each insect individually under a dissecting microscope and placed in a buffer containing beta-alanine and acetic acid, and stored for later production processing.

Each species of HV is assessed for content of hyaluronidase and phospholipase activity. For the preparation of some venoms, multiple species are mixed to represent the major stinging insect species across the United States. There are several species that are required for the YJ (*Vespula*) and wasp (*Polistes*) venoms, but

availability may lead to variation in additional species used in these mixes. Mannitol is added to the venoms before freeze-drying as an osmotic buffer to help stabilize the venom protein.

As for stinging ants, the only extracts available for clinical use are WBEs. In contrast to the other Hymenoptera whose WBE contained little or no venom allergens, the WBE of imported fire ants has shown adequate activity for diagnostic testing and immunotherapy. Fire ant venoms are superior but have not been practical to produce. Immunotherapy for allergy to Australian Jack Jumper ants (genus *Myrmecia*) is performed using venom extract that is locally produced.²¹

Part 3: VIT

Having fulfilled the necessary criteria (history of an anaphylactic reaction to a Hymenoptera sting and confirmatory venom-specific IgE), VIT was recommended. Because specific IgE was identified by either *in vitro* or skin testing methods for YJ, yellow-faced hornet, and wasp, and after discussing the risks, benefits, and alternatives with the patient, VIT for wasp and mixed vespids was initiated. Using the protocol outlined in the package insert, the patient progressed through the build-up phase with several large local reactions significant enough to require minor dose adjustments in building up to the final maintenance dose of 300 µg for mixed vespid and 100 µg for wasp. She had a single systemic reaction with generalized urticaria, hoarse voice, and anxiety immediately after achieving a full maintenance dose. A 50% reduction in dose, followed by a resumption with increasing doses, resulted in ultimately reaching and safely receiving a full maintenance dose of VIT.

Clinical utilization of venom extracts

Venom immunotherapy. In clinical use, the properties and characteristics of the venom extract products are closely tied to the benefits, risks, doses, and regimens of VIT. The clinical protection is dependent on the activity of all the allergens in the extract. Reduced protection with HB VIT in some patients may be due to reduced activity of Api m 10 in HB venom extracts.²² Reduced protection with YJ or *Polistes* wasp VIT may be due to the absence of certain species in these mixes. Additional purification steps in the production of the extracts lead to reduced adverse effects with VIT.¹⁶

Venom extract production. Once the venoms have been collected and processed, they may need to be mixed and standardized before being prepared for final commercial packaging. Of the 6 HV products marketed in the United States, 3 contain a single species (HB, yellow hornet, and white-faced hornet) and 3 are mixtures of species.

The commercially available extracts used for testing and treatment for *Vespula* (YJ) allergy in North America consist of mixtures of venom from multiple YJ species but some may vary. Although *V. squamosa* has been included in the commercially available YJ venom extract mixes since 1980, it highlights the reality that all species may not be included in all commercial mixes. This could result in missed diagnostic and therapeutic opportunities of YJ venom—allergic patients. Mixed vespid venom includes the YJ species mix as well as 2 species of *Dolichovespula* (yellow hornet and white-faced hornet) in equal proportions (100 µg/mL of each, for a total of 300 µg/mL).

Similar to *Vespula* venom extracts, the commercially available extracts of *Polistes* (paper wasp) venom in North America are mixes from 3 to 5 species; venoms from most of the 21 North

American species are not included. The *Polistes* species are phylogenetically closely related and as such these mixtures are believed to provide adequate diagnostic and therapeutic clarity in most cases of hypersensitivity.

There are many other species of *Vespula* and *Polistes* that people may encounter, but those in the venom extracts are known to cause the most stings. The most common species in other countries, such as in Europe, vary somewhat from those in the United States but are antigenically homologous. The one known exception is the Mediterranean wasp, *Polistes dominula*, which is prevalent in southern Europe and has invaded much of the United States. The venom of *P. dominula* has incomplete cross-reactivity with other *Polistes* species venom; in endemic areas, commercial *Polistes* venom extracts may fail to diagnose or treat patients allergic to *P. dominula* (Mediterranean wasp).²³ This venom is used as a single species for VIT in some areas of Europe but is not included in any commercial *Polistes* venom.²⁴

HVs are standardized for phospholipase and hyaluronidase content. In the case of HB venom, this provides a real measure of the major allergen, with more assurance of homogeneity among batches and manufacturers. Vespid venoms are not standardized for the major allergen (which has no enzymatic activity). Although there is not known to be significant variability, the actual content of Ves v 5 is not established by any standard, nor is it reported for each lot of venom.

Part 4: Venom stability, sterility, shortages

Having achieved a protective maintenance dose, she was able to gradually increase the interval between venom injections to 6 weeks and then 8 weeks, as indicated in the Practice Parameters.¹² She did well for nearly 3 years, subsequently extending the interval up to 12 weeks and requiring occasional dose adjustments for missed shots. Unfortunately, there was a disruption in venom supply for VIT, requiring a switch in manufacturer. The composition of the commercial venoms is similar, but because differences exist, dose adjustments were initially made. She continued to maintain her active outdoor lifestyle, completed a second pregnancy, and with exception of local discomfort demonstrated no systemic symptoms following a field sting by a suspected YJ.

Postproduction issues. For almost 40 years, commercially available venom extracts for the testing and treatment have come from 2 suppliers, ALK-Abello (originally from Pharmacia) and Jubilant HollisterStier (as they are currently known). As summarized in Table 1, the products produced were very similar in venom content, but not identical. As this case illustrates, minor dosing adjustments may be prudent when treatment is changed from one supplier to another.

The handling, storage, mixing, dilution, and clinical use of venom extracts in practice are described in the product package insert. As supplied from the manufacturer, the lyophilized extract must be reconstituted to a concentration of 100 µg/mL using a diluent containing 0.03% HSA. The purpose of this specific diluent is to provide stabilization of protein allergens and minimize adsorption to the vials walls, which is particularly important for more diluted extracts. HSA has also been shown to partially protect the proteins from the deleterious effects of phenol, which also becomes more of a concern at greater dilutions. HSA performs the function served by the glycerin used as a preservative in

TABLE I. Commonly available venom tests for HV sensitization

Genus	Common name	No. of species in North America	ALK extracts	HollisterStier extracts	Phadia immunoCAP
<i>Apis</i>	Honeybee	1	<i>Apis mellifera</i>	<i>Apis mellifera</i>	<i>Apis mellifera</i>
<i>Bombus</i>	Bumblebee	46	0	0	<i>Bombus terrestris</i>
<i>Vespa</i>	Hornet	1	0	0	<i>Vespa crabro</i>
<i>Dolichovespula</i>	Hornet (Aerial YJ)	5	<i>D arenaria</i> <i>D maculata</i>	<i>D arenaria</i> <i>D maculata</i>	<i>D arenaria</i> <i>D maculata</i>
<i>Vespula</i>	Yellow jacket	13	1 mix of 6 species	1 mix of 5 species	1 mix of 6 species
<i>Polistes</i>	Wasp (paper wasp)	21	1 mix of 5 species	1 mix of 4 species	

many environmental and food allergen extracts. It is extremely rare that the HSA component itself would cause any adverse effect, but some individuals or groups may refuse products that contain human blood proteins. For such individuals, reconstituting the venom with an alternative diluent may not give optimal stabilization, but if it is used immediately, the venom dose should be adequate and is certainly better than nontreatment. When reconstituting the venom, it is recommended to avoid shaking the vial or agitating the solution in any way that would cause foaming of the solution, because this indicates some degradation of the proteins. This foaming is caused by any proteins in solution, but is increased by the addition of albumin.

Freeze-dried extracts show minimal deterioration over the shelf life period. After reconstitution to 100 µg/mL, venom extracts show some reduction in potency over time, dependent on the species. Yellow hornet (*D arenaria*) shows the most noticeable rate of loss (of phospholipase A activity) and is labeled with a 6-month shelf life. Other venoms will retain their activity for up to 12 months (as indicated in the package labeling). The reconstituted venom may be diluted for treatment or for skin testing, usually in 10-fold dilutions to 10, 1, 0.1, 0.01, or 0.001 µg/mL. The diluted solutions may lose activity more rapidly than the stock extract, so the guidance in the package insert suggests that the shelf life is 6 to 12 months from the date of reconstitution for the 100 µg/mL concentration (according to the product label), 1 month (from the date of dilution) for concentrations of 1.0 to 10 µg/mL, and 14 days from the date of dilution for 0.1 µg/mL. They suggest that lower concentrations should be made fresh daily. Dilutions should always be made from stock extracts (100 µg/mL) and not from previously diluted extracts. There have been no published reports on the shelf life and stability of HV extracts. Shelf lives for skin test concentrations as listed in the product package inserts (since they were introduced in 1979) were based on conservative estimates in association with the Food and Drug Administration (FDA). Kelly et al²⁵ reported (in the abstract), on the basis of skin test responses in 10 patients, that “diluted venoms displayed remarkable stability, maintaining 100% of their original potency after storage for 2, 4 and 6 weeks. Potency diminished 0-6% by 8 weeks and 7-13% with 10 or 12 weeks storage. No significant difference was observed in stability results among the 5 venom species or different concentrations stored.”

Mixing of venom extracts of different insect genera for testing, or treatment, is not recommended. This is primarily due to concerns about protease activity and degradation of venom

protein allergens. Most venom allergens, and many of the non-allergen components, have enzymatic activity. The mixing of the YJ and hornet venoms was found to be no problem, presumably because they are phylogenetically and allergenically nearly identical. For lack of evidence of nondegradation, it is not recommended to mix *Vespula* (YJ) with *Polistes* (paper wasp) venom or HB venom.

These are general recommendations, but there are limited data on the stability and activity of individual venom allergens in the commercial extracts. For example, if only 1 of the allergens loses activity rapidly, only those individuals who are exclusively or primarily sensitized to that component would be affected by this loss of activity. There would likely be no reduction in activity in the mean skin test results for a group of patients if only 1 or 2 in the group have such a component-specific sensitivity.

Sterility/infection control. Sterility controls are carried out at all stages of the final production process. This is very similar to all other allergen products except that there is a final lyophilization step. Sterility controls include the use of ISO Class 5 clean rooms until vials have been lyophilized, stoppered, and sealed. All finished products are subjected to sterility tests using FDA-validated procedures per USP Chapter 71. Final release of final venom lots also requires FDA approval after their inspection of batch records and can include at their discretion, retesting the product in their laboratories. USP-compliant container closure components are selected and validated for multientry use. Phenol in the diluent is required by FDA for any product that is intended for multiple entry use.

Shortages. On several occasions in recent years there have been shortages of venom extract products in the US market. This can involve a temporary backorder of a specific species due to either collection or production problems that cause an interruption in the supply chain. On occasion there can be manufacturing problems that create a more prolonged interruption in the supply from a single manufacturer for 6 to 12 months. In such situations, there is a need for patients to be treated with venoms from a different supplier. As discussed earlier, there has often been concern about possible differences between the venom extracts from different laboratories and the potential clinical consequences, but to date switching suppliers appears to be safe.²⁶ The characteristics of the venom extracts and the available published literature on the clinical use of HV served as the evidence base for a guiding document for clinicians

during the most recent shortages.²⁷ Unfortunately, as of 2018, there is a single supplier of venom in the United States, so any interruption in supply will lead to an interruption or denial of treatment for thousands of patients who are at risk for insect sting anaphylaxis.

ADDITIONAL CONSIDERATIONS

This case illustrates several additional relevant situations in which the characteristics of HV may affect diagnosis and treatment. First, that the role of mast cell disease and its association with venom-allergic individuals have assumed new prominence in recent years. The relationship is discussed in more detailed elsewhere.²⁸ However, renewed understanding was sufficient to prompt a change in clinical recommendation regarding the measurement of serum tryptase in venom-allergic individuals.¹² Second, for various reasons, the culprit insect is often not known with certainty. In cases in which the history and physical examination provide an exceedingly high level of confidence of a specific insect, such as imported fire ant or HB, focused testing may be sufficient diagnostically. However, when the confidence is not high, testing with all relevant venoms is warranted. With its high level of sensitivity, venom skin testing remains the initial testing of choice. However, in a clinical situation of low confidence in insect identification, additional *in vitro* testing may also be warranted to species negative on skin tests, and treatment with all sensitized venoms is initiated.

Unlike standard immunotherapy to aeroallergen where the dosing intervals are variable, but relatively close at every 1 to 4 weeks, with VIT the interval may be much longer extending to every 6 to 12 weeks. As such, doses are often missed or delayed. In addition, recognizing the life-threatening nature of venom allergy compared with standard aeroallergen immunotherapy accelerated build-up regimens such as cluster, rush, or ultrarush may be clinically warranted especially for high-risk individuals. Although there is no established standard or recommendation, the dose adjustment illustrated in this case appears to be a safe and effective management strategy. In Europe, the dose regimen is similar to that in North America. The interval between maintenance doses is most commonly every 4 weeks during the first year of treatment, increasing to 6 weeks in the second year and every 8 weeks from years 3 to 5. For individuals on extended treatment, 12 weeks is recommended.

In addition to minor dosing differences between Europe and North America, there are other notable differences in management. As noted, in North America, recent recommendation for the management of cutaneous systemic reactions in adults has moved away from VIT treatment. Recent European Guidelines²⁹ recommend treatment with VIT for quality-of-life reasons. Other differences include the availability and common use of recombinant venoms and basophil activation tests for diagnosis, regional differences in relevant insects, as well as the availability of bumblebee extract for diagnosis and treatment in some counties. Perhaps the most clinically relevant difference is the availability of purified aqueous extract and purified aluminum hydroxide-adsorbed preparations used as depo products. All are effective, but unfortunately, not approved by the US FDA and thereby unavailable to the US market. Other differences in the management of the venom-allergic individuals between Europe and North America are outlined elsewhere, and may vary on the basis of country.

SUMMARY

Venom allergy is the prototypic model for the diagnosis and management of anaphylaxis. The initial exposure is generally obvious, the progress of reactions predictable, and diagnostic methods reliable and readily available. Unlike other causes of anaphylaxis, preventative treatment is highly effective and available. Treatment with VIT provides an important window into our understanding of the management strategy of immunotherapy. HVs are the prototypic standardized allergens and their reliability supports their clinical use for diagnosis and prevention of insect sting anaphylaxis. Storage, sterility, stability, and product mixing of venoms are important considerations for allergists who care for venom-allergic individuals. Recent availability of recombinant venom allergens has brought to light potential deficiencies in venom extracts that may reduce their accuracy for diagnosis and efficacy for treatment. In recent years, concerns about venom shortages have emerged and adaptive management strategies were recommended. Even more recently, ALK has exited the venom market in North America and a single supplier of venoms, Jubilant HollisterStier, remains. These events in supplier changes that have occurred may present their own challenges, but for the moment supplies for venom testing and treatment appear to be adequate and stable.

Acknowledgments

We thank Derek Constable, PhD (Technical Director, Jubilant HollisterStier Allergy Products Division), for providing details and guidance on the technical aspects of venom production. Additional background information on venom collection and production was provided by Greg Plunkett, PhD (ALK-Abello Inc).

REFERENCES

1. Golden DB. Insect sting allergy and venom immunotherapy: a model and a mystery. *J Allergy Clin Immunol* 2005;115:439-47.
2. Lichtenstein LM. Insect allergy: the state of the art. *J Allergy Clin Immunol* 1979;64:5-12.
3. Benton AW, Morse RA, Stewart JD. Venom collection from honeybees. *Science* 1963;142:228-30.
4. 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.
5. King TP, Sobotka AK, Kochoumian L, Lichtenstein LM. Allergens of honey bee venom. *Arch Biochem Biophys* 1976;172:661-71.
6. Loveless MH, Fackler WR. Wasp venom allergy and immunity. *Ann Allergy* 1956;14:347-66.
7. Benson R, Semenov H. Allergy in its relation to bee sting. *J Allergy Clin Immunol* 1930;1:105-11.
8. American Academy of Allergy Insect Allergy Committee. Insect sting allergy, cooperative study. *JAMA* 1965;193:115-20.
9. Golden DB. Large local reactions to insect stings. *J Allergy Clin Immunol Pract* 2015;3:331-4.
10. Bilo BM, Bonifazi F. Epidemiology of insect-venom anaphylaxis. *Curr Opin Allergy Clin Immunol* 2008;8:330-7.
11. 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.
12. Golden DBK, Demain J, Freeman T, Graft D, Tankersley M, Tracy J, et al. Stinging insect hypersensitivity: a practice parameter update 2016. *Ann Allergy Asthma Immunol* 2017;118:28-54.
13. Akre RD, Greene A, MacDonald JF, Landolt PJ, Davis HG. Yellowjackets of America North of Mexico. Washington, DC: US Department of Agriculture; 1980.
14. Hoffman DR, Dove DE, Moffitt JE, Stafford CT. Allergens in Hymenoptera venom, XXI: cross-reactivity and multiple reactivity between fire ant venom and bee and wasp venoms. *J Allergy Clin Immunol* 1988;82:828-34.

15. King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. *Int Arch Allergy Immunol* 2000;123:99-106.
16. Bilo MB, Severino M, Cilia M, Pio A, Casino G, Ferrarini E, et al. The VISYT trial: venom immunotherapy safety and tolerability with purified vs nonpurified extracts. *Ann Allergy Asthma Immunol* 2009;103:57-61.
17. 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;102:47-50.
18. Vos B, Kohler J, Muller S, Stretz E, Rueff F, Jacob T. Spiking venom with rVes v 5 improves sensitivity of IgE detection in patients with allergy to *Vespula* venom. *J Allergy Clin Immunol* 2013;131:1225-7.
19. Blank S, Seismann H, Michel Y, McIntyre M, Cifuentes L, Braren I, et al. Api m 10, a genuine *A. mellifera* venom allergen, is clinically relevant but under-represented in therapeutic extracts. *Allergy* 2011;66:1322-9.
20. King TP, Sobotka AK, Alagon A, Kochoumian L, Lichtenstein LM. Protein allergens of white faced hornet, yellow hornet, and yellow jacket venoms. *Biochemistry* 1978;17:5165-74.
21. Brown SG, Wiese MD, Blackman KE, Heddle RJ. Ant venom immunotherapy: a double-blind placebo-controlled crossover trial. *Lancet* 2003;361:1001-6.
22. Frick M, Fischer J, Helbling A, Ruëff F, Wiczorek D, Ollert M, et al. Predominant Api m 10 sensitization as risk factor for treatment failure in honeybee venom immunotherapy. *J Allergy Clin Immunol* 2016;138:1663-71.
23. Blanca M, Garcia F, Miranda A, Carmona MJ, Garcia J, Fernandez J, et al. Determination of IgE antibodies to *Polistes dominulus*, *Vespula germanica*, and *Vespa crabro* in sera of patients allergic to vespids. *Allergy* 1991;46:109-14.
24. Severino M, Bonadonna P, Bilo MB, Cortellini G, Mauro M, Schiappoli, et al. Safety and efficacy of immunotherapy with *Polistes dominulus* venom: results from a large Italian database. *Allergy* 2009;64:1229-30.
25. Kelly D, Meister A, Golden DBK, Kagey-Sobotka A, Norman PS, Hamilton RG, et al. Stability of Hymenoptera venom skin test extracts [abstract]. *J Allergy Clin Immunol* 2003;111:S333.
26. Ridolo E, Martignago I, Passalacqua G, Mauro M, Incorvaia C. Evaluation of the safety of a protocol for switching venom immunotherapy products. *Ann Allergy Asthma Immunol* 2018;120:429-30.
27. Golden DB, Bernstein DI, Freeman TM, Tracy JM, Lang DM, Nicklas RA. AAAAI/ACAAI Joint Venom Extract Shortage Task Force Report. *J Allergy Clin Immunol Pract* 2017;5:330-2.
28. Bonadonna P, Zanotti R, Muller U. Mastocytosis and insect venom allergy. *Curr Opin Allergy Clin Immunol* 2010;10:347-53.
29. Sturm GJ, Varga EM, Roberts G, Mosbech H, Bilo MB, Akdis CA, et al. EAACI guidelines on allergen immunotherapy: Hymenoptera venom allergy. *Allergy* 2018;73:744-64.