

CME review

The sting of the honeybee: an allergic perspective

Tyson C. Brown, MD,* and Michael S. Tankersley, MD*

Objective: To provide a focused understanding of the uniqueness and special considerations of honeybee allergy.

Data Sources: A PubMed search using the keywords *honeybee*, *allergy*, and *hypersensitivity* yielded the initial relevant articles. Additional significant sources cited in the reference lists of the initial articles were also used.

Study Selection: More than 130 articles were reviewed, and the most relevant references were selected for inclusion in this article.

Results: The honeybee differs from other flying Hymenoptera from both an entomologic and allergic standpoint. The entomology literature is not often consulted by the allergist when addressing avoidance of honeybees. Beekeepers are a particular population at risk for honeybee exposure and allergy. Venom composition, sting mechanism, diagnostic evaluation, and immunotherapy efficacy and safety all have unique considerations specific to the honeybee.

Conclusions: Honeybee is a significant cause of venom hypersensitivity. By understanding unique behaviors of honeybees, proper avoidance measures may be addressed with patients. Honeybee venom is complex, and the delivery mechanism provides for a large but often variable amount of injected venom. Diagnosis of honeybee allergy by imperfect skin and serologic testing further complicated by cross-reactivity is often difficult. Generally, honeybee immunotherapy is less safe and less effective than for other flying Hymenoptera. Efforts to improve testing and immunotherapy are under way.

Ann Allergy Asthma Immunol. 2011;xx:xxx.

Off-label disclosure: Drs Brown and Tankersley have indicated that this article does not include the discussion of unapproved/investigative use of a commercial product/device.

Financial disclosure: Drs Brown and Tankersley have indicated that in the last 12 months they have not had any financial relationship, affiliation, or arrangement with any corporate sponsors or commercial entities that provide financial support, education grants, honoraria, or research support or involvement as a consultant, speaker's bureau member, or major stock shareholder whose products are prominently featured either in this article or with the groups who provide general financial support for this CME program.

Instructions for AMA PRA Category 1 Credit™:

1. Read the CME review article in this issue carefully and complete the activity by answering the self-assessment examination questions on the CME evaluation form on page XXX.
2. To receive CME credit, submit the completed CME evaluation form to the ACAAI office prior to December 31, 2012.

INTRODUCTION

Apis mellifera, commonly known as the honeybee, has played a major agricultural role throughout history. Not native to the United States, they are thought to have been brought from Europe during colonization in the 1600s. In 1925, honeybees were responsible for the first observations, testing, and immu-

nomodulatory vaccination of Hymenoptera allergy.¹ Since then, strides have been made in understanding this particular Hymenoptera with elucidation of various properties of insect behavior, venom specifics, and methods for diagnosis and immunotherapy that make it unique among its order.

This review aims to provide a focused understanding of the uniqueness and special considerations of honeybee allergy. A PubMed search using the keywords *honeybee*, *allergy*, and *hypersensitivity* yielded the initial relevant articles. Additional significant sources cited in the reference lists of the initial articles were also used. More than 130 articles were reviewed, and the most relevant references were selected for inclusion in this article. Practical summary points for each section of this review are provided in [Table 1](#).

Affiliations: * Department of Medicine, Allergy/Immunology Division, San Antonio, Texas.

Funding Sources: This study was funded by the US Air Force.

Received for publication August 8, 2011; Received in revised form September 21, 2011; Accepted for publication September 22, 2011.

© 2011 American College of Allergy. Published by Elsevier Inc. All rights reserved.

doi:10.1016/j.anai.2011.09.015

Table 1. Practical Take-Home Points by Section

Entomology

- Most honeybee stings occur in the warmer months from agitating a single bee engaged in foraging.
- Some volatile substances in common foods, polishes, and cosmetic products may mimic alarm pheromones, thus eliciting defensive behavior.
- Abrupt movement and dark clothes heighten defensive awareness in bees.
- Africanized honeybees, as opposed to the more common European honeybees, are usually responsible for mass envenomations.

Epidemiology

- Risk of a honeybee sting at some point in one's life may be as high as 25% to 30%.
- Fatalities and systemic reactions may be more often associated with honeybee stings than with stings from other flying Hymenoptera.
- Children tend to have lower systemic reaction rates than adults.
- Interestingly, sensitivity to honeybee venom may be lower than other flying Hymenoptera in adults but appears to be higher in children.

Beekeepers

- Beekeepers are at higher risk for sting-elicited systemic reactions due to increased number of stinging events; however, more than 200 stings per year correlates with protection from systemic reactions.
- Beekeepers are not seen by the allergist often and, possibly as a result, seem to be less informed about sting risk and epinephrine use, thus representing a high-risk population.

Venom Allergens and Composition

- Api m 1 (phospholipase A₂) is the most potent allergen in honeybee venom and appears to complement the anaphylactic process by increasing leukotriene production.
- Api m 2 (hyaluronidase) is the second most potent honeybee venom allergen and shares substantial sequence identity with hyaluronidase of other flying Hymenoptera.
- Api m 4 (melittin) is not a potent allergen but is mainly responsible for pain and hemolysis.
- Many other enzymes and toxins make up much of the honeybee venom and are also responsible for toxicity reactions, especially in mass envenomations.

Sting Mechanism and Venom Delivery

- Honeybee sting apparatus has multibarbed lancets that lead to autotomy or separation from the abdomen.
- The amount of venom in the sac ranges from 59 to 141 $\mu\text{g/mL}$ on average with wide intracolony and intercolony variation.
- After a stinging event, quickly removing the stinger may minimize a reaction because most venom is delivered within the first 5 to 10 seconds; however, if the stinger is left in the skin for more than 30 seconds, all of the venom can be assumed to be injected.

Diagnostic Tools

- Honeybee extract for skin testing and immunotherapy is standardized to Api m 1 (phospholipase A₂).
- Because of its high sensitivity (94%-100%), intradermal skin testing (to a concentration of 1 $\mu\text{g/mL}$ of honeybee extract) is the standard diagnostic tool in combination with a history that suggests a systemic reaction to a sting.
- Serologic CAP honeybee specific IgE is useful for diagnostic purposes, with sensitivities higher than 90%.
- Cross-reacting carbohydrate determinants, hyaluronidase, and other components may account for significant false double-positivity among flying Hymenoptera, often clouding the diagnostic picture.
- Other testing modalities are less established and controversial.

Immunotherapy

- Honeybee immunotherapy provides approximately 75% to 85% protection from future stings, which is lower than the approximately 85% to 93% protection seen with yellow jacket immunotherapy.
- Risk for systemic reaction to an injection with extract during the full course of honeybee immunotherapy ranges from 24% to 41% vs 5% to 25% for other flying Hymenoptera.
- A maintenance dose of 50 μg may be as efficacious as the standard 100- μg dose; however, prospective studies are needed for confirmation.
- Some patients may require doses up to 400 μg to prevent systemic reactions to field stings.
- As with other flying Hymenoptera, spacing the maintenance dose intervals to 3 months appears to be safe and effective.

On the Horizon

- Component-resolved diagnostics may help clarify difficult double-positive patients in the future to better tailor immunotherapy.
- Removal of cross-reacting carbohydrate determinants from recombinant Api m 2 for diagnosis may also clarify the double-positive patients.

ENTOMOLOGY

Honeybees thrive in colonies that number in the tens of thousands, with one queen in each colony that produces eggs. The queen may live up to 2 years; however, workers have an approximately 6-week lifespan. Honeybees are divided into hive workers, nurses, guards, soldiers, and foragers. The larger (up to 9-fold) and older bees are usually the guards, soldiers, and foragers and are typically responsible for encounters with humans.² Honeybees communicate and organize the workforce via chemical (ie, pheromones), tactile, vibratory, visual, and/or auditory signals. Foragers can stray far from the hive for pollen or nectar, often up to several miles. It is this activity in which the bee is most often engaged when encountered by a human and a sting occurs. Interestingly, foraging (as with other bee tasks) is usually accomplished as a stereotypic and circadian activity, meaning the foraging honeybee will often appear at the same group of flowers at about the same time of day. Generally, foraging occurs in the spring and summer, when there are flowers in bloom and when temperatures are higher than 60°F. Honeybee activity, therefore, is typically minimal in the winter months.²

Understanding the entomology of honeybee foraging and defensive behavior is important for allergists when attempting to realistically address sting avoidance.³ Unfortunately, the entomologic literature is not frequently consulted by allergists when addressing avoidance.⁴ Allergists often advise patients to not “look or smell like a flower” in their counseling in an effort to prevent honeybee attraction, despite lack of evidence supporting this recommendation. A honeybee forager’s flower recognition comes with learning to associate and integrate a series of complex cues that involve color, shape, scent, and location and usually includes a food reward.^{4,5} Flower recognition also involves light in the UV spectrum not perceived from or produced by everyday human activity or clothing.⁴ Although no study to date has quantitatively evaluated common human use of various foods, polishes, varnishes, and cosmetic products as a risk for honeybee sting, some evidence exists supporting a link between certain volatile substances found in these products and venom alarm pheromones (ie, pheromones causing defensive behavior, such as isopentyl acetate and other volatile hydrocarbons released from the glands from both the honeybee and the sting apparatus after separation).^{6,7}

Honeybee stings occur from individual foraging workers or whole colonies.⁴ In most cases, a single honeybee is encountered unintentionally by direct interaction, such as stepping on the bee, disturbing an open beverage container, or agitating the bee during yard or garden work. Such proximal disturbance clearly elicits a defensive response, which increases the risk for sting, although compared with other flying Hymenoptera, honeybees are relatively docile. Evidence shows that meteorologic factors, such as elevated air temperature, warm, dry seasons, and time of day (ie, early morning and late evening), may also increase the risk for sting.⁸ Furthermore, abrupt, proximal movement (usually within a few feet), dark objects (ie, a human with dark clothes

or a bear), and mammalian breathing (to include humans) heighten defensive awareness and increase the risk for sting.^{4,6} Typically, these less well-known agitators are what stir a colony, which is alerted via alarm pheromones from the guards and soldiers. After a sting, the stinger usually remains in the person because of multiple small barbs, and incidentally, the honeybee continues to pursue and harass the intruder by flying around the face until the bee dies or the attack is over. After losing the stinger, the honeybee will typically die within hours or days but not immediately.⁶

The honeybee response to an attack depends on the severity of the threat, the size of the colony, and the type of honeybee. European honeybees (EHBs; ie, *Apis mellifera mellifera* or *Apis mellifera ligustica*) and more recently Africanized honeybees (AHBs; ie, a hybrid of EHB and the African honeybee, *Apis mellifera scutellata*) are common species found in the United States. Often EHBs require the threat to be within a few meters of the colony before eliciting a response compared with being within 100 m or more for AHBs. Typically, individuals sustain tens to hundreds of stings for EHBs vs hundreds to thousands for AHBs, and once the individual has retreated 50 m from the colony, the EHBs cease pursuit, whereas the AHBs may continue chasing for up to several kilometers.^{4,6} The EHBs and AHBs are equally important for pollination and differ most notably in aggression.⁹

EPIDEMIOLOGY

Epidemiologic data specifically addressing honeybee stings are rare. Although prevalence of stings from all flying Hymenoptera bees ranges from 56.6% to 85.5% in an adult life,¹⁰⁻¹² with one recent study showing the prevalence in children to be 37.5% through 13 years of age,¹³ no study addresses honeybee alone. These adult studies, mainly in Europe, however, report that approximately one-third of the stings were due to honeybee (suggesting up to 25%-30% prevalence) compared with other vespids. The annual incidence of all stings was found to be 22.6% in one recent study from Turkey, with honeybees accounting for 38.8% of the last reported sting.¹⁰ No study exists solely looking at the annual incidence of honeybee stings. However, research showing the decrease in honeybee stings may be of interest in light of colony collapse, a mysterious phenomenon of near or complete disappearance of entire honeybee colonies.¹⁴ Fatalities from stings due solely to honeybee have not been studied. One recent report, however, from Costa Rica recorded 52 deaths during a 22-year period or 0.74 deaths per year per 1 million inhabitants due “mainly to bee stings,”¹⁵ which is significantly greater than 0.14 deaths per year per 1 million reported previously in the United States for all stings by flying Hymenoptera.¹⁶ Interestingly, this same US study showed that approximately half of fatalities resulted from honeybee stings. The lifetime prevalence of a systemic reaction (SR) to Hymenoptera ranges from 0.9% to 3.3% in adults, with one study noting that 45.5% of their 1.54% SR rate was due to honeybee.^{11,17,18} In only 2 studies, children appear to have a lower SR prevalence of 0.2% to 1.3%, with no study delineating honeybee.^{13,19} Prevalence of large local reactions in both adults and children vary widely

(3.1%-26.4%),¹⁸⁻²¹ but no data exist separating honeybees from other Hymenoptera.

Honeybee sensitivity as measured by the presence of serum specific IgE or by positive skin test result has been observed in several studies. Overall prevalence of sensitivity to flying Hymenoptera in all adults as measured by either one or both testing methods showed rates of 9.3% to 26.5%.^{18,20-22} Honeybee specific IgE was found in 3.7% to 10% of all adults;^{18,21,22} whereas a sample from Australia showed 16% honeybee sensitivity in an adult population of 3429 patients.²⁰ One study addressed children and showed that overall 3.66% of children tested positive to any flying Hymenoptera by skin testing, of which 81% of the positive test results were due to honeybee.¹⁹ This finding suggests that compared with adults children are substantially more often sensitized by honeybee, which is possibly explained by activity differences between the age groups.

BEEKEEPERS

Beekeepers represent a unique population of those affected by honeybee allergy. Because of their frequent exposure and higher sting rate, they are thought to be at higher risk for honeybee allergy. Epidemiologic data suggest this may be true when compared with the general population because beekeepers show higher SR rates of 4.4% to 38% and local reaction rates of 12% to 76%.²³⁻²⁵ On average, a beekeeper has a reported 57 stings per year.²⁵ Of note, protection correlates with receiving more than 200 stings per year; however, 50 stings per year may also provide benefit. Beekeepers receiving fewer than 25 stings per year had a high SR rate of 45%.²³ When compared with the general population, beekeepers were found to have lower levels of venom specific IgE, lower skin sensitivity, and higher levels of venom specific IgG but no difference in the severity of SRs.^{23,26,27} Müller²⁸ analyzed much of the literature and concluded that elevated risk for a SR was found in beekeepers with initial stings within the first years of beekeeping (particularly in the spring), fewer than 10 annual bee stings, history of atopic disease, and symptoms of upper respiratory tract infection during work with beehives. Interestingly, a recent survey of British beekeepers suggests that female sex, positive family history for honeybee allergy, more than 2 years of beekeeping before the first SR, and use of antihistamine premedication before hive

attendance predisposed individuals to SRs.²⁴ In this study, of those individuals who reported being stung and having a reaction, only 16.6% were seen by an allergy specialist and 18% carried an epinephrine autoinjector. Although much of these data suggest that beekeepers who have been in the profession for many years and who are frequently stung might have some protection from serious reaction, it seems more evident that beekeepers might be a less informed, underserved, high-risk population that should continue to warrant special attention.

VENOM ALLERGENS AND COMPOSITION

For nearly 40 years honeybee venom components have been extensively studied. Known and defined allergens are summarized in Table 2. The most potent allergen is phospholipase A₂ (PLA₂) (also known as Api m 1).²⁹ In addition to the IgE response it elicits, its actions on the human immune system may enhance the immediate immune response. PLA₂ is a glycoprotein enzyme composed of 134 residues that causes hydrolysis of phospholipids and leads to a potentially significant increase in arachidonic acid. An elevation in arachidonic acid level leads to increased leukotriene production (leukotrienes C₄, D₄, and E₄) by the 5-lipoxygenase pathway.³⁰ Release of these mediators is known to cause increased mucous production, bronchoconstriction, and vascular permeability, thus complementing the anaphylactic mediators. Honeybee PLA₂ has significant sequence identity with bumble bee (ie. *Bombus terrestris*) PLA₂ but no similarity with the major vespid allergen PLA₁.³¹

Hyaluronidase (Api m 2) acts to break up the connective-tissue matrix, allowing other venom components to spread into the tissue,³² and is the second most potent allergen. Hyaluronidase is found in most Hymenoptera venoms and has 55% sequence identity with its vespid counterpart, which may account for most of its known venom cross-reactivity.³¹ Melittin (Api m 4) is unusually small compared with all other Hymenoptera allergens, makes up most honeybee venom by weight, is the main molecule responsible for pain and inflammation of the sting, and can cause red blood cell lysis at high concentrations, as experienced in patients with multiple stings.³³

Some honeybee venom components have functions, as described above, that act directly on the tissue, whereas others activate these proteins. Other content found within the venom in

Table 2. Defined Honeybee Venom Allergens^a

Allergen name	Biochemical name	Size, kDa	Venom, %	IgE positive, %	Allergen potency
Api m 1	Phospholipase A ₂	16	7-15	97	Very high
Api m 2	Hyaluronidase	39	0.5-1.5	?	Moderate
Api m 3	Acid phosphatase	43	1	37	?
Api m 4	Melittin	3	35-50	28-50	Low
Api m 5	Dipeptidylpeptidase IV	100	0.5	?	?
Api m 6	Cysteine-rich trypsin inhibitor	8	1-2	42	?
Api m 7	CUB serine protease	39	?	80	?
Api m 8	Carboxylesterase	70			
Api m 9	Serine carboxylesterase	60			
Api m 10	Icarapin	50-55			

^a Data are from www.allergen.org. Question mark indicates unknown.

small quantities that may account for both local and systemic effects include various neurotoxins (apamin, tertiapin), neurotransmitters (noradrenaline), vasoactive compounds (histamine and dopamine), and mast cell degranulators (mast cell degranulating protein and tertiapin). Other carbohydrates, free amino acids, and small organics, such as pheromones, also exist in the venom sac.³³ In mass stinging incidents large amounts of these enzymes and toxins may cause hemolysis, rhabdomyolysis, thrombocytopenia, acute renal failure, hepatitis, mental status changes, and cardiac arrest. Mass envenomations are more common with the honeybee and typically involve stings in the hundreds to thousands, owing to the larger colony sizes than those of other Hymenoptera.³²

STING MECHANISM AND VENOM DELIVERY

The honeybee stinger apparatus, a modified ovipositor, is composed of 1 guide stylet and 2 multibarbed, hollow lancets that slide along the guide stylet, all attached to functional muscles and a venom sac. A channel, created between the intersection of these 3 components, guides venom flow. Reciprocating, rhythmic movement of the lancets along the stylet, anchored by the lancets' barbs, buries the stinger deeper into the tissue. This rhythmic, motor-like action begins automatically with stinger apparatus denervation caused by separation from the abdomen or autotomy. Movement of the lancets pushes the venom distally by means of umbrella-like valves attached to the channel side of the lancets. As the stinger drives farther in, venom is excreted into the ever-deepening tissue layers; the entire process is completed within approximately 30 seconds.³⁴

Timing and quantity of venom released during stings have been studied. Schumacher et al³⁵ found that there are significant intracolony and intercolony differences in the amount and type of venom components in the EHB and AHB venom sacs. On average, 134.1 μg of EHB venom and 98.2 μg of AHB venom by dry weight were found in their respective venom sacs. These numbers coincided with a follow-on study by Schumacher et al,³⁴ which observed extrusion of 141.9 μg of EHB venom, on average, after sting. Both studies showed greater venom amounts than the 59 μg average previously reported³⁶ and generally accepted. However, both studies also noted wide variation (SD, ± 53 -89 μg) in volumes among individual EHBs. Interestingly, PLA₂ accounted for a greater concentration in AHBs vs EHBs with the converse apparent for melittin. The second study showed that within approximately 5 to 10 seconds a substantial amount of venom was delivered, and all or nearly all the venom sac contents were emptied and approximately two-thirds of the stinger embedded within the tissue after 30 seconds. These results suggest that if the stinger is removed as quickly as possible, especially from a sensitized individual, a reaction may be minimized based on a lesser venom volume being injected.^{34,35} This observation was supported by a later study showing smaller wheals correlating with shorter times that the stinger was left in the skin. Interestingly, this study did not show a statistical difference in wheal size with regard to method of stinger removal (scraping vs pinching), somewhat contradict-

ing the common perception that care must be taken with stinger removal so as to not compress the sac.³⁷

DIAGNOSTIC TOOLS

An evaluation for honeybee sensitivity should be pursued after historical evidence of a SR. In North America, skin testing and serologic IgE measurements are the 2 most commonly used tools when assessing sensitivity to any Hymenoptera. Because patient history has poor reliability when distinguishing which Hymenoptera venom to test, it is recommended to test for all 5 standardized venoms.³⁸ Unlike vespid and wasp, which are standardized to hyaluronidase concentrations, honeybee venom extract used for diagnostic purposes and immunotherapy is standardized to the major allergen Api m 1 (PLA₂).³⁹ Intradermal skin testing is considered the standard to confirm diagnosis, although venom extract package inserts recommend starting with epicutaneous skin testing. Intradermal starting concentrations are usually 0.001 $\mu\text{g}/\text{mL}$ and progress to either 0.1 $\mu\text{g}/\text{mL}$ or 1 $\mu\text{g}/\text{mL}$, depending on the desired sensitivity. Honeybee venom is generally considered to be more irritating to the skin than other Hymenoptera venoms at similar concentrations, although no study has been designed to specifically compare honeybee to other Hymenoptera. In an early study by Patrizzi et al⁴⁰ looking specifically at honeybee venom skin testing compared with clinical history, sensitivity and specificity using a cutoff of 0.1 $\mu\text{g}/\text{mL}$ were 94% and 97%, respectively, and changed to 100% and 71%, respectively, when the cutoff was increased to 1 $\mu\text{g}/\text{mL}$. A later, larger study of more than 220 patients with honeybee allergy demonstrated similar results with purported marked increase in specificity from 1 to 0.1 $\mu\text{g}/\text{mL}$ with little change in sensitivity.⁴¹ Most believe, however, that allergic patients may be missed because of lower sensitivity, so 1 $\mu\text{g}/\text{mL}$ is generally considered a more appropriate maximal intradermal concentration,^{38,42} in line with other Hymenoptera venoms. Of note, it has been shown that some patients with a convincing clinical history of an anaphylactic reaction to Hymenoptera do not demonstrate positive *in vitro* or *in vivo* test results.⁴³ Skillful clinical judgment in these particular patients will be required when deciding therapeutic options.

Generally, serologic honeybee venom specific IgE levels agree with skin test results. However, these levels are usually less sensitive. In the study of honeybee testing by Patrizzi et al,⁴⁰ based on clinical history, a radioallergosorbent test serum antigen specific IgE level of 0.35 kU/L or higher produced a sensitivity of 73% with a specificity of 94%.⁴⁰ More recent and relevant serologic techniques using CAP for detection of serologic honeybee specific IgE, compared with skin testing, were found to have a sensitivity as high as 98% with any positive level (≥ 0.35 kU/L) but a specificity of only 58%.⁴⁴ Another larger study, based on clinical history, showed CAP specific IgE sensitivity and specificity to honeybee to be 92.5% and 83.7%, respectively.⁴⁵ Of note, although CAP honeybee specific IgE tends to have a similar sensitivity to that of vespids, the specificity tends to be markedly lower.^{44,45}

As mentioned previously, hyaluronidase and other molecules have potentially significant cross-reactivity between the

honeybee and other flying Hymenoptera.^{46,47} This cross-reactivity may cloud an otherwise simple clinical scenario when, for example, a patient has clearly been stung once, yet shows positivity to 2 flying Hymenoptera (ie, double-positivity). Cross-reacting carbohydrate determinants (CCDs) are recurring epitopes found on glycoproteins within pollens, foods, and venoms to which the body has been shown to make specific IgE. CCDs may also account for venom double-positivity, which seems to be especially true for honeybee allergic patients, who, compared with yellow jacket and wasp allergic patients, were more often falsely double-positive due to CCDs.^{48,49}

Other areas of evaluation are being explored. One study compared standard honeybee venom to recombinant PLA₂ in both skin testing and serologic testing and found greater sensitivity in the natural bee venom but improved specificity in the recombinant PLA₂.⁵⁰ A live sting challenge has been proposed as a viable diagnostic tool for honeybee in select patients choosing not to initiate immunotherapy to determine loss of sensitivity over time⁵¹; however, this proposal remains controversial.⁵² Other *in vitro* tests, such as histamine release, basophil activation as measured by flow cytometry for CD63 expression, and leukotriene release evaluated by enzyme-linked immunosorbent assay, continue to be revisited and improved on as diagnostic tools,^{45,53} and although some show promise, particularly in separating double-positivity, they continue to be difficult to perform and to standardize.

IMMUNOTHERAPY

Ultimately, the goal of immunotherapy is to provide protection from future stings. Since honeybee venom replaced whole-body extract as the primary material for honeybee immunotherapy, successful protection has improved significantly. However, honeybee immunotherapy appears to be less effective at providing future protection when compared with other flying Hymenoptera. Using 100- μ g maintenance doses of honeybee venom extract for at least 3 years, then either conducting a sting challenge or evaluating field stings 0 to 10 years after cessation of the immunotherapy, SR rates ranged from 15.8% to 23%.⁵⁴⁻⁵⁶ Comparatively, SR rates after sting challenge and/or field stings 1 to 7 years after at least a 3-year yellow jacket immunotherapy course using 100- μ g doses ranged from 7.5% to 13.5%.⁵⁵⁻⁵⁷ Interestingly, in a landmark study inclusive of all flying Hymenoptera, Golden et al⁵⁸ showed that an average of 18 years after completion of a 3.5-year venom immunotherapy course in children, moderate to severe SRs occurred in 5% compared with 32% of those not receiving immunotherapy.⁵⁸ Although this study did not distinguish honeybee from other flying Hymenoptera, it provided a basis with which to compare the effectiveness of venom immunotherapy over time.

Honeybee immunotherapy also has a higher SR rate when compared with other flying Hymenoptera. Again, using 100 μ g as a maintenance target, the SR rate for all patients during immunotherapy (regardless of buildup schedule) ranged from 24.1% to 41% for honeybee and 5.2% to 25% for other flying Hymenoptera.^{55,59,60} One of these studies reported the SR rate

per injection to be 4.6% for honeybee vs 2.3% for the common wasp.⁵⁹ Comparing these studies may have some limitations because SR definitions may vary and immunotherapy protocols differ. Differences between SR rates for accelerated vs conventional buildup schedules are well known and are consistent for all flying Hymenoptera.³⁸

Although 100- μ g maintenance dosing is generally accepted as optimal,³⁸ some debate still continues regarding appropriate dosing for honeybee immunotherapy. Two retrospective studies regarding SR rates over the long term using honeybee venom immunotherapy suggest a 50- μ g maintenance dose may be adequate for protection from future honeybee stings. Reisman and Livingston⁶¹ used 50- μ g dosing and reported a 2.7% SR rate (3/108 patients) to field stings occurring from 1 month to 8 years after initiation of immunotherapy.⁶¹ Although only 20% of these patients had honeybee allergy, 2 of the 3 SRs occurred in these honeybee allergic patients, suggesting the SR rate for honeybee is higher than the reported overall SR rate. The second study recently published by Houliston et al⁶² showed a SR rate of 13.6% 1 to 18 years after completion of 2 to 7 years of immunotherapy using a 50- μ g maintenance dose.⁶² Both of these studies are comparable with current 100- μ g maintenance dosing protection for the honeybee described earlier. In the other direction, one retrospective review found that those continuing to react to sting challenge during honeybee immunotherapy benefitted from increasing the maintenance dose stepwise up to 400 μ g by showing either resolution of or milder SRs.⁶³

It is generally recommended that maintenance injections for all Hymenoptera should be given every 4 to 6 weeks.³⁸ However, initial studies mainly using the honeybee and confirmed later with all flying Hymenoptera showed that spacing to a 12-week interval may be safe and effective.^{64,65} Two other studies looked to extend the honeybee immunotherapy maintenance dose to 6-month intervals. One failed to extend the interval after showing a SR in 3 of 8 monosensitized patients (38%) on sting challenge.⁶⁶ In contrast, the other study showed spacing may be reasonable with a SR in 5 of 20 honeybee-only allergic patients (25%), with 4 of 5 being Mueller grade 0 to 1 reactions.⁶⁷ The current recommendation of honeybee immunotherapy remains 3 to 5 years, which is similar to all other Hymenoptera, with some requiring life-long therapy for severe reactions.³⁸

Although conventional subcutaneous immunotherapy remains the mainstay for its proven efficacy, other methods and adjuncts have been and are being explored. Sublingual immunotherapy using honeybee venom was shown recently to reduce large local reactions,⁶⁸ although significant doubt exists as to its clinical relevance at preventing future SRs.⁶⁹ Passive immunotherapy using beekeeper γ -globulin, particularly in patients prone to frequent SRs with conventional honeybee immunotherapy, has been shown to be safe and effective in this select group.⁷⁰ Its use should be limited because it may be costly and only provide temporary protection.⁷¹ Another option that has been reported for honeybee

allergic patients who are difficult to treat is the adjunctive use of omalizumab as pretreatment, although reports of both success and failure have been made.^{72,73}

ON THE HORIZON

Component-resolved diagnostics has allowed further elucidation of various venom components of the honeybee and other Hymenoptera.⁷⁴ Although testing has not been approved by the Food and Drug Administration in the United States, component-resolved diagnostics shows great promise, particularly in difficult diagnostic conundrums and decision points for starting immunotherapy in the setting of double-positive patients. Also, recent advances in recombinant technology have shown that by removing CCDs from the Api m 2 allergen, cross-reactivity is significantly decreased and options for diagnosis and therapy become more clear.⁷⁵ Detection of specific IgE to these CCDs may also help eliminate confusion in double-positive honeybee allergic patients in the future.⁴⁹

CONCLUSION

The honeybee remains important for our society from both an agricultural and allergic perspective. By understanding the honeybee's attractants, offenders, and colony defensive behaviors, we may better guide patients' future avoidance measures. The honeybee also is unique in its venom composition, and because of this, some diagnostic and immunotherapeutic challenges exist. As the difficulties in honeybee hypersensitivity are elucidated, our understanding of the honeybee will continue to be shaped, and our knowledge will be furthered in the study of venom allergy.

REFERENCES

- Braun LIB. Notes on desensitization of a patient hypersensitive to bee stings. *S Afr Med Rec.* 1925;23:408–409.
- Bloch G. The social clock of the honeybee. *J Biol Rhythms.* 2010;25:301–317.
- Buckingham RB, Levine MI. Protective measures against insect stings and bites. In: Levine MI, Lockey RF, eds. *Monograph on Insect Allergy*. 4th ed. Pittsburgh, PA: Dave Lambert Associates; 2003:153–154.
- Greene A, Breisch NL. Avoidance of bee and wasp stings: an entomological perspective. *Curr Opin Allergy Clin Immunol.* 2005;5:337–341.
- Weinstock GM, Robinson GE, Gibbs RA. Insights into the social insects from the genome of the honeybee *Apis mellifera*. *Nature.* 2006;443:931–949.
- Breed MD, Guzman-Novoa E, Hunt GJ. Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. *Annu Rev Entomol.* 2004;49:271–298.
- Ono M, Terabe H, Hori H, Sasaki M. Components of giant hornet alarm pheromone. *Nature.* 2003;424:637–638.
- Woyke J. Diurnal and seasonal variation in defensive behavior of African bees *Apis mellifera adansonii* in Ghana. *Apidologie.* 1992;23:311–322.
- McKenna WR. Killer bees: what the allergist should know. *Pediatr Asthma Allergy Immunol.* 1992;6:275–285.
- Onbasi K, Onbasi O, Eminbeyli L, Kaynak C. Prevalence and alternative therapy methods for bee and wasp allergy in Van. *Allergy.* 2008;63:246–247.
- Charpin D, Birnbaum J, Lanteaume A, Vervloet D. Prevalence of allergy to Hymenoptera stings in different samples of the general population. *J Allergy Clin Immunol.* 1992;90:331–334.
- Incorvaia C, Mauro M, Pastorello EA. Hymenoptera stings in conscripts. *Allergy.* 1997;52:680–681.
- Jennings A, Duggan E, Perry JJ, Hourihane JOB. Epidemiology of allergic reactions to Hymenoptera stings in Irish school children. *Pediatr Allergy Immunol.* 2010;21:1166–1170.
- Ratnieks FLW, Carreck NL. Clarity on honey bee collapse? *Science.* 2010;327:152–153.
- Prado M, Quiros D, Lomonte B. Mortality due to Hymenoptera stings in Costa Rica, 1985–2006. *Pan Am J Public Health.* 2009;25:389–393.
- Barnard JH. Studies of 400 Hymenoptera sting deaths in the United States. *J Allergy Clin Immunol.* 1973;52:259–264.
- Prado M, Vega A, Munoz E, Moreno-Ancillo A. Epidemiologic observations on *Hymenoptera* allergy in Spain: the alergologica-2005 study. *J Invest Allergol Clin Immunol.* 2009;19:51–55.
- Golden DBK, Marsh DG, Kagey-Sobotka A, et al. Epidemiology of insect venom sensitivity. *JAMA.* 1989;262:240–244.
- Novembre E, Cianferoni A, Bernardini R, et al. Epidemiology of insect venom sensitivity in children and its correlation to clinical and atopic features. *Clin Exp Allergy.* 1998;28:834–838.
- Stuckey M, Cobain T, Sears M, Cheney J, Dawkins RL. Bee venom hypersensitivity in Busselton. *Lancet.* 1982;320:41.
- Fernandez J, Blanca M, Soriano V, Sanchez J, Juarez C. Epidemiological study of the prevalence of allergic reactions to Hymenoptera in a rural population in the Mediterranean area. *Clin Exp Allergy.* 1999;29:1069–1074.
- Bjornsson E, Janson C, Plaschke P, Norrman E, Sjoberg O. Venom allergy in adult Swedes: a population study. *Allergy.* 1995;50:800–805.
- 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;73:332–340.
- Richter AG, Nightingale P, Huissoon AP, Krishna MT. Risk factors for systemic reactions to bee venom in British beekeepers. *Ann Allergy Asthma Immunol.* 2011;106:159–163.
- Munstedt K, Hellner M, Winter D, von Georgi R. Allergy to bee venom in beekeepers in Germany. *J Invest Allergol Clin Immunol.* 2008;18:100–105.
- Eich-Wanger C, Muller U. Bee sting allergy in beekeepers. *Clin Exp Allergy.* 1998;28:1292–1298.
- Light WC, Reisman RE, Wypych JJ, Arbesman CE. Clinical and immunological studies of beekeepers. *Clin Allergy.* 1975;5:389–395.
- Müller U. Bee venom allergy in beekeepers and their family members. *Curr Opin Allergy Clin Immunol.* 2005;5:343–347.
- King TP, Sobotka AK, Kochoumian L, Lichtenstein LM. Allergens of honey bee venom. *Arch Biochem Biophys.* 1976;172:661–671.
- Mustafa FB, Ng FS, Nguyen TH, Lim LH. Honeybee venom secretory phospholipase A2 induces leukotriene production but not histamine release from human basophils. *Clin Exp Immunol.* 2008;151:94–100.
- King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. *Int Arch Allergy Immunol.* 2000;123:99–106.
- Vetter RS, Visscher PK, Camazine S. Mass envenomations by honey bees and wasps. *West J Med.* 1999;170:223–227.
- Hoffman DR. Hymenoptera venoms: composition, standardization, stability. In: Levine MI, Lockey RF, eds. *Monograph on Insect Allergy*. 4th ed. Pittsburgh: Dave Lambert Associates; 2003:37–53.
- Schumacher MJ, Tveten MS, Egen NB. Rate and quantity of delivery of venom from honeybee stings. *J Allergy Clin Immunol.* 1994;93:831–835.
- Schumacher MJ, Schmidt JO, Egen NB, Dillon KA. Biochemical variability of venoms from individual European and Africanized honeybees (*Apis mellifera*). *J Allergy Clin Immunol.* 1992;90:59–65.
- Hoffman DR, Jacobson RS. Allergens in Hymenoptera venom XII. *How much protein is in a sting?* *Ann Allergy.* 1984;52:276–278.
- Visscher PK, Vetter RS, Camazine S. Removing bee stings. *Lancet.* 1996;348:301–302.
- Golden DBK, Moffitt J, Nicklas RA. Stinging insect hypersensitivity: a practice parameter update 2011. *J Allergy Clin Immunol.* 2011;127:852–854.

39. Golden DBK. Diagnostic methods in insect allergy. In: Levine MI, Lockey RF, eds. *Monograph on Insect Allergy*. 4th ed. Pittsburgh, PA: Dave Lambert Associates; 2003:63–74.
40. Patrizzi R, Muller U, Yman L, Hoigne R. Comparison of skin tests and RAST for the diagnosis of bee sting allergy. *Allergy*. 1978;34:249–256.
41. Day JH, Buckeridge DL, Welsh AC. Risk assessment in determining systemic reactivity to honeybee stings in sting-threatened individuals. *J Allergy Clin Immunol*. 1994;93:691–705.
42. Georgitis JW, Reisman RE. Venom skin tests in insect-allergic and insect-nonallergic populations. *J Allergy Clin Immunol*. 1985;76:803–807.
43. Golden DBK, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Insect sting allergy with negative venom skin test responses. *J Allergy Clin Immunol*. 2001;107:897–901.
44. Leimgruber A, Lantin J-P, Frei PC. Comparison of two *in vitro* assays, RAST and CAP, when applied to the diagnosis of anaphylactic reactions to honeybee or yellow jacket venoms. *Allergy*. 1993;48:415–420.
45. Scherer K, Weber JM, Jermann TM, et al. Cellular *in vitro* assays in the diagnosis of Hymenoptera venom allergy. *Int Arch Allergy Immunol*. 2008;146:122–132.
46. Reisman RE, Muller UR, Wypych JI, Lazell MI. Studies of coexisting honeybee and vespid-venom sensitivity. *J Allergy Clin Immunol*. 1984;73:246–252.
47. Reisman RE, Wypych JI, Lazell MI. Further studies of patients with both honeybee- and yellow-jacket-venom-specific IgE. *Int Arch Allergy Appl Immunol*. 1987;82:190–194.
48. Muller UR, Johansen N, Petersen AB, Fromberg-Nielson J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespa* venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. *Allergy*. 2009;64:543–548.
49. Erzen R, Korosec P, Silar M, Music E, Kosnik M. Carbohydrate epitopes as a cause of cross-reactivity in patients allergic to Hymenoptera venom. *Wein Klin Wochenschr*. 2009;121:349–352.
50. Muller U, Fricker M, Wymann D, Blaser K, Cramer R. Increased specificity of diagnostic tests with recombinant major bee venom allergen phospholipase A2. *Clin Exp Allergy*. 1997;27:915–920.
51. Goldberg A, Confino-Cohen R, Mekori YA. Deliberate Hymenoptera sting challenge as a diagnostic tool in highly selected venom-allergic patients. *Ann Allergy Asthma Immunol*. 1995;75:30–32.
52. Dubois AEJ. Investigational and clinical use of the sting challenge. *Curr Opin Allergy Clin Immunol*. 2003;3:283–285.
53. Sturm GJ, Bohm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W. The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. *Allergy*. 2004;59:1110–1117.
54. Muller U, Berchtold E, Helbling A. Honeybee venom allergy: results of a sting challenge 1 year after stopping successful venom immunotherapy in 86 patients. *J Allergy Clin Immunol*. 1991;87:702–709.
55. Muller U, Helbling A, Berchtold E. Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. *J Allergy Clin Immunol*. 1992;89:529–535.
56. Lerch E, Muller UR. Long-term protection after stopping venom immunotherapy: results of re-stings in 200 patients. *J Allergy Clin Immunol*. 1998;101:606–612.
57. Golden DBK, Kwiterovich KA, Kagey-Sobotka A, Lichtenstein LM. Discontinuing venom immunotherapy: extended observations. *J Allergy Clin Immunol*. 1998;101:298–305.
58. Golden DBK, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Outcomes of allergy to insect stings in children, with and without venom immunotherapy. *N Engl J Med*. 2004;351:668–674.
59. Kalogeromitros D, Makris M, Koti I, et al. A simple 3-day “rush” venom immunotherapy protocol: documentation of safety. *Allergol Immunopathol*. 2010;38:69–73.
60. Birnbaum J, Charpin D, Vervloet D. Rapid Hymenoptera venom immunotherapy: comparative safety of three protocols. *Clin Exp Allergy*. 1993;23:226–230.
61. Reisman RE, Livingston A. Venom immunotherapy: 10 years of experience with administration of single venoms and 50 mg maintenance doses. *J Allergy Clin Immunol*. 1992;89:1189–1195.
62. Houliston L, Nolan R, Noble V, et al. Honeybee venom immunotherapy in children using a 50-mg maintenance dose. *J Allergy Clin Immunol*. 2011;127:98–99.
63. Rueff F, Wenderoth A, Przybilla B. Patients still reacting to a sting challenge while receiving conventional Hymenoptera venom immunotherapy are protected by increased venom doses. *J Allergy Clin Immunol*. 2001;108:1027–1032.
64. Goldberg A, Confino-Cohen R. Maintenance venom immunotherapy administered at 3-month intervals is both safe and efficacious. *J Allergy Clin Immunol*. 2001;107:902–906.
65. Cavallucci E, Ramondo S, Renzetti A, et al. Maintenance venom immunotherapy administered at a 3-month interval preserves safety and efficacy and improves adherence. *J Invest Allergol Clin Immunol*. 2010;20:63–68.
66. Goldberg A, Confino-Cohen R. Effectiveness of maintenance bee venom immunotherapy administered at 6-month intervals. *Ann Allergy Asthma Immunol*. 2007;99:352–357.
67. Baenkler HW, Meusser-Storm S, Eger G. Continuous immunotherapy for Hymenoptera venom allergy using six months intervals. *Allergol Immunopathol*. 2005;33:7–14.
68. Severino MG, Cortellini G, Bonadonna P, et al. Sublingual immunotherapy for large local reactions caused by honeybee sting: a double-blind, placebo-controlled trial. *J Allergy Clin Immunol*. 2008;122:44–48.
69. Rueff F, Bilo MB, Jutel M, Mosbech H, Muller U, Przybilla B. Sublingual immunotherapy with venom is not recommended for patients with Hymenoptera venom allergy. *J Allergy Clin Immunol*. 2009;123:272–273.
70. Lessof MH, Sobotka AK, Lichtenstein LM. Effects of passive antibody in bee venom anaphylaxis. *Johns Hopkins Med J*. 1978;142:1–7.
71. Muller UR, Morris T, Bischof M, Friedli H, Skarvil F. Combined active and passive immunotherapy in honeybee-sting allergy. *J Allergy Clin Immunol*. 1986;78:115–122.
72. Galera C, Soohun N, Zankar N, Caimmi S, Gallen C, Demoly P. Severe anaphylaxis to bee venom immunotherapy: efficacy of pretreatment and concurrent treatment with omalizumab. *J Invest Allergol Clin Immunol*. 2009;19:225–229.
73. Gomis VS, Delgado PG, Hernandez EN. Failure of omalizumab treatment after recurrent systemic reactions to bee-venom immunotherapy. *J Invest Allergol Clin Immunol*. 2008;18:225–226.
74. de Graaf DC, Aerts M, Danneels E, Devreese B. Bee, wasp, and ant venomomics pave the way for a component-resolved diagnosis of sting allergy. *J Proteomics*. 2009;72:145–154.
75. Seismann H, Blank S, Braren I, et al. Dissecting cross-reactivity in Hymenoptera venom allergy by circumvention of α -1,3-core fucosylation. *Mol Immunol*. 2010;47:799–808.

Requests for reprints should be addressed to:
 Tyson C. Brown, MD
 Department of Medicine
 Allergy/Immunology Division
 2200 Bergquist Dr, Suite 1
 San Antonio, TX 78236
 E-mail: tyson.brown@us.af.mil