Stinging Insect Allergy

A Clinician's Guide

Theodore M. Freeman
James M. Tracy
Editors

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Editors
Theodore M. Freeman
San Antonio Allergy and Asthma Clinic
San Antonio, Texas
USA

James M. Tracy
Allergy, Asthma & Immunology
Associates P.C.
Omaha, Nebraska
USA

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Foreword

I have been fascinated with insects for almost as long as I can remember. I can distinctly recall only one writing assignment from third grade and that is because it was about a heroic ant who saved his nest from certain destruction. It was somewhat later that I learned that my hero was most likely a heroine since the overwhelming majority of individuals in any hymenoptera colony are female. Much later in life I began to develop an interest in medical entomology, and I learned I had a *Homo sapiens*-centric viewpoint of the world. Did you know that there are an estimated 10 quintillion (10, 000,000,000,000,000,000) individual insects alive at any time and that the total biomass of ants alone is nearly equivalent to that of humans?[1, 2] The more I learned, the more I developed a fascination and interest with insects.

When I became an allergy-immunology fellow, it seemed only natural that I focus my research on hymenoptera, especially ants. This avocation eventually led to me being asked to edit this volume.

This book is focused squarely on hymenoptera as these are clearly the most important species for the allergist-immunologist and in many ways is a descendant of the *Monograph on Insect Allergy*.[3] My coeditor Dr. Jim Tracy and I believe in the importance of giving depth to the clinical aspects of hymenoptera stings by examining areas outside these strict medical aspects; consequently there are chapters on the entomologic aspects of hymenoptera and the history of hymenoptera stings in humans. In addition we believe that some families of hymenoptera deserve special attention such as bees and ants; therefore, we have included chapters devoted to these insects. Every allergist-immunologist that has a clinical practice knows that our patients expect us to be the experts on all

[1] https://www.si.edu/Encyclopedia_SI/nmnh/buginfo/bugnos.htm

[2] https://en.wikipedia.org/wiki/Biomass_(ecology

[3] Levin MI and Lockey RF, eds. Monograph on Insect Allergy Fourth Ed. Dave Lambert Associates, Pittsburgh, PA. 2003

insects and not just those that sting. So we include chapters on biting insects, mosquitos, and insects as vectors of other infectious diseases. Finally and not surprisingly, the more we learn, the more questions we have about these insects. Dr. Tracy wrote a chapter reviewing some of the most important questions that we continue to face.

I hope you will find this guide not only useful but interesting and enlightening. I do, but then I always have.

San Antonio, TX, USA Theodore M. Freeman

Contents

Part I

1 History of Hymenoptera Allergy 3
James M. Quinn

2 Entomological Aspects of Insect Sting Allergy 17
Robert S. Jacobson

3 Hymenoptera Venoms: Toxicity, Components, Stability, and Standardization 43
Ellen L. Danneels, Matthias Van Vaerenbergh, and Dirk C. de Graaf

4 Clinical Aspects of Hymenoptera Allergy: IgE Response, Stings, and Immunotherapy 71
Kevin M. White

5 Diagnostic In Vivo and In Vitro Methods in Insect Allergy 85
Robert G. Hamilton

6 Pathologic Findings in Hymenoptera Sting Fatalities 101
Jessica B. Perkins and Anne B. Yates

7 Treatment of Hymenoptera Reactions: Acute and Prophylactic 109
David F. Graft

8 Adverse Reactions to Skin Testing and Immunotherapy with Hymenoptera Venoms and Whole-Body Extracts 125
Robbie Pesek and Richard F. Lockey

9 Duration of Venom Immunotherapy 141
David B.K. Golden

Part II

10 Unique Aspects of Bee Allergy and Reactions 155
William R. McKenna and Tyson C. Brown

11 The Imported Fire Ant: The US Experience 189
Mike Tankersley and Daniel Steigelman

12 Mosquito Hypersensitivity: Clinical Updates. 203
Renata J.M. Engler and Howard C. Crisp

13 Reactions to Arthropod Bites. 231
John C. Carlson

14 Arthropods and Human Disease 247
Michael O'Connell

Part III

15 Behavior, Ecology, Natural History, and Distribution of Stinging Hymenoptera 271
Justin O. Schmidt

16 Insect Sting Allergy and Mast Cell Disease 285
Patrizia Bonadonna, Roberta Zanotti, and Franziska Ruëff

17 Insect Allergy: Unanswered Questions, a Postscript 307
James M. Tracy

Index. 313

List of Contributors

Patrizia Bonadonna Allergy Unit, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy

Tyson C. Brown Department of Medicine, Landstuhl Regional Medical Center, Landstuhl, Germany

John C. Carlson Sections of Allergy and Immunology and Community Pediatrics and Global Health, Tulane University School of Medicine, New Orleans, LA, USA

Howard C. Crisp North Texas Allergy and Asthma Associates, Dallas, TX, USA

Ellen L. Danneels Department of Biochemistry and Microbiology, Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Ghent, Belgium

Renata J.M. Engler Department of Medicine, Allergy-Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Department of Medicine, Walter Reed National Military Medical Center (WRNMMC), Bethesda, MD, USA

Theodore M. Freeman San Antonio Allergy and Asthma Clinic, San Antonio, TX, USA

David B.K. Golden Johns Hopkins University, Baltimore, MD, USA

Dirk C. de Graaf Department of Biochemistry and Microbiology, Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Ghent, Belgium

David F. Graft Department of Asthma and Allergic Diseases, Park Nicollet Clinic, Minneapolis, MN, USA

Robert G. Hamilton Division of Allergy and Clinical Immunology, Departments of Medicine and Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Johns Hopkins Asthma and Allergy Center, Johns Hopkins Dermatology, Allergy and Clinical Immunology (DACI) Reference Laboratory, Baltimore, MD, USA

Robert S. Jacobson Senior Technical Specialist (Retired), Greer Laboratories Inc., Lenoir, NC, USA

Richard F. Lockey Division of Allergy and Immunology, Department of Internal Medicine, University of South Florida Morsani College of Medicine, Tampa, FL, USA

William R. McKenna Department of Medicine, University of Texas Health Science Center San Antonio, San Antonio, TX, USA

Michael O'Connell Division of Pulmonology, Allergy, Critical Care, and Sleep Medicine, Department of Medicine, Ohio State University College of Medicine, Columbus, OH, USA

Jessica B. Perkins Department of Pediatrics, University of Mississippi Medical Center, Jackson, MS, USA

Robbie Pesek University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock, AR, USA

James M. Quinn Department of Allergy and Immunology, San Antonio Uniformed Services Health Education Consortium, Wilford Hall Ambulatory Surgical Center, San Antonio, TX, USA

Franziska Ruëff AllergieZENTRUM, Klinik und Poliklinik für Dermatologie und Allergologie, Klinikum der Universität München, München, Germany

Justin O. Schmidt Southwestern Biological Institute, Tucson, AZ, USA

Department of Entomology, University of Arizona, Tucson, AZ, USA

Daniel Steigelman Department of Allergy and Immunology, San Antonio Uniformed Services Health Education Consortium, Wilford Hall Ambulatory Surgical Center, San Antonio, TX, USA

Mike Tankersley The University of Tennessee Health Science Center, Memphis, TN, USA

James M. Tracy Division of Allergy and Immunology, Department of Internal Medicine, Creighton University School of Medicine, Omaha, NE, USA

Allergy, Asthma and Immunology Associates, P.C., Omaha, NE, USA

Matthias Van Vaerenbergh Department of Biochemistry and Microbiology, Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Ghent, Belgium

Kevin M. White Department of Allergy, US Air Force, RAF Lakenheath, Brandon, UK

Anne B. Yates Department of Pediatrics, University of Mississippi Medical Center, Jackson, MS, USA

Roberta Zanotti Section of Hematology, Department of Medicine, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy

Part I

Chapter 1
History of Hymenoptera Allergy

James M. Quinn

In the Beginning

Among insects, the origins of the order hymenoptera date back hundreds of millions of years [1]. Within the last 100 million years, the aculeate clade of hymenoptera developed modified ovipositors used for stinging [1, 2]. The aculeate clade includes the families of the common and medically relevant stinging insects of today, namely, the Apidae (bees), Vespidae (wasps, yellow jackets, hornets), and Formicidae (ants) [2].

The origins of the first written record of possible stinging insect allergy remain controversial. Many medical authors attribute the first account of death from anaphylaxis to a wasp sting suffered by the Egyptian Pharaoh, King Menes, circa 2600 BC [3–5]. This account originated from a publication by L. A. Waddell in 1930 in which he interpreted hieroglyphics from an alleged burial site for King Menes. Waddell's account included the interpretation that the fatal sting from the "wolfish insect of the field," referring to the wasp, occurred on a voyage to the "Lake of the Peak in Urani Land," referring to the British Isles or specifically Ireland [6]. However, this account has been convincingly challenged for decades [7, 8]. In 1969, Chafee asked two respected Egyptologists to review Waddell's work [7].

The opinions and assertions contained herein are the private views of the author and are not to be considered as official or reflecting the views of the Department of Defense or other departments of the US Government.

J.M. Quinn, M.D.
Department of Allergy and Immunology, San Antonio Uniformed Services Health Education Consortium, Wilford Hall Ambulatory Surgical Center, San Antonio, TX, USA
e-mail: james.quinn.11@us.af.mil

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One of the experts found that Waddell had made an error by changing the interpretation of a hieroglyphic from a hippopotamus to a wasp. The other expert outlined a broader controversy that questioned the existence of King Menes as anything other than a mythical figure. In 2004, Krombach and colleagues reviewed Waddell's work and leveled similar criticisms [8]. They found that historical scholars widely viewed Menes as a mythical figure or as a misidentified figure confused with earlier monarchs or a composite of myth, legend, and reality. They also raised significant questions regarding the accuracy of Waddell's interpretation of the hieroglyphics. Finally, Krombach and colleagues cited other competing accounts of the demise of King Menes at the hands of hippopotami, dogs, or crocodiles; he even noted an account whereby King Menses was rescued from his dogs by a crocodile. Regardless of the uncertain origins of the entertaining tale of King Menes, there are ancient written accounts of death due to individual stings from flying insects that likely represent anaphylaxis. Accounts from the Babylonian Talmud of such events date back approximately 2000 years [1, 9]. In the passing millennia, there have been sporadic descriptions of fatalities resulting from a single or a limited number of flying hymenoptera stings. These descriptions often did not attempt to attribute pathophysiologic causation or simply categorized the deaths as anomalies or curiosities of medicine [1, 10].

Era of Discovery

Around the turn of the twentieth century, many important discoveries in allergy and immunology were made. Some of these discoveries were quickly applied to patients suffering from stinging insect allergy. Earlier accounts attributed the cause of systemic reactions to varied theories such as "particular susceptibility" to venom toxin or direct introduction of toxin, toxic bacilli, or pollen into superficial veins [1, 11–14]. A turning point in the understanding and categorization of stinging insect reactions began with the description of anaphylaxis in dogs by Portier and Richet in 1902 [15]. In 1914, Waterhouse applied the knowledge discovered by Portier and Richet when he described a patient who had been a beekeeper earlier in life but many years later suffered repeated rapid and dramatic systemic reactions [16]. He described two events resulting in "… lost consciousness … feeble pulse … difficulty breathing … sudden urticaria …" as a result of isolated stings, two on the hand at one occasion and one on the ear at a different occasion. Initially, he referred to this case as "nothing especially remarkable" given the existing descriptions of similar events in the literature of the time. However, Waterhouse made the important observation—"one is struck by the resemblance between these serious effects and the typical symptoms of anaphylactic shock in animals." He further observed that the events "cannot be accounted for by the mere absorption of a minute quantity of irritant poison" and put forth the "supposition" that "the extraordinary increase in his sensitiveness" was due to having "been previously sensitized by former stings."

Work by Freeman and Noon in the early twentieth century described the successful treatment of allergic rhinoconjunctivitis with immunotherapy [17, 18]. Freeman and Noon utilized a series of subcutaneous injections of increasing doses from a crude extraction of grass pollen. They utilized conjunctival challenge for the diagnosis and as a measure of therapeutic efficacy. Meanwhile, Schloss adapted von Pirquet's method of skin testing for tuberculosis to investigate methods to evaluate patients with food anaphylaxis [19]. Schloss published a reproducible method for identifying "toxic" foods in order to avoid the dangers of actual feeding of the suspected food. Schloss described abrading the skin in a 1–2 mm diameter circle followed by "gently rubbed" application of dilutions of the desired testing substances. Within 5–15 min, he described the development of urticarial wheals of 1.5–3.0 cm in diameter and 1–5 mm in elevation. He further characterized the reactions as always immediate followed by disappearance in 0.5–1 h, and he documented the absence of the reaction with several negative control substances. In concert with the work of Freeman, Noon, and Schloss, L.I.B. Braun reported a case of the evaluation and desensitization of a patient with hypersensitivity to bee stings [20]. The patient reported progressively worsening symptoms of anaphylaxis after seven episodes spanning 9 years. Braun described defining the patient's sensitivity by skin testing. He detailed the procedure of snipping off the posterior one-eight inch of the body of a bee, grinding it up, extracting it in saline, and repeatedly filtering the solution. He assessed the patient's sensitivity by applying various dilutions of the crude extract to one-eight inch squared scarified areas of the skin. He tested for sensitivity by applying one drop of the crude extract to the area. Within 8 min, the patient had the onset of a local wheal and flare as well as the onset of a systemic reaction resulting in generalized urticaria, angioedema, "suffocating cough," generalized tremor, and tachycardia. Her condition "became rather alarming" ultimately leading to the administration of "10 minims of adrenalin" with resultant gradual improvement. Subsequently, Braun undertook efforts to desensitize the patient. Beginning with "a very high dilution" of the original extract, he gradually increased the doses. Initially he applied increasing concentrations to scarified areas and progressed to injections of increasing concentrations and amounts of the undiluted extract. After reaching 1.5 cm^3 of undiluted extract, he performed a sting challenge resulting only in "extremely mild" symptoms and found it "quite unnecessary to inject adrenalin."

The Whole-Body Extract Era

In the late 1920s and 1930s, studies began to appear that applied increasing sophistication and evidence-based scientific approach to stinging insect allergy. In 1929, the work of Benson and Semenov on bee sting allergy was presented at the Seventh Annual Meeting of the Association for the Study of Allergy. In 1930, they published their remarks from the meeting as an original article "Allergy in its relation to bee sting" in the first volume of *The Journal of Allergy* [21]. Their work summarized

much of the published medical literature regarding bee stings and reactions including attempts at chemical characterization of the venom, characterization and speculative pathophysiology of the mechanisms for reactions, as well as description of the recent (at the time) reports of testing for sensitivity and of treatment options. They added their own original research demonstrating that venom contained histamine or a histamine-like imidazole in small quantities. Their conclusion was that the histamine contained in the venom of a single sting was unlikely the cause of any reaction beyond the local site but left room for uncertainty. In the paper, Benson and Semenov further discounted prior speculation, namely, by Gibb, that allergic reactions from bee stings might be the result of pollen carried by bees. Their initial position was that "the etiologic importance of such a relationship is … overrated …." Their subsequent microscopic examination of the stinging apparatus found it "almost entirely free from contamination with a pollen" and concluded that the possibility that pollens would produce anaphylaxis from a sting "is at best remote …." In addition, they separately skin tested with a crude extract of the venom, the stinging apparatus, and the whole body. They applied 1:100 dilutions to a 3 mm superficial scratch and measured wheal size classifying the reaction on a 1–4+ scale. They included appropriate positive and negative control reagents and negative control patients. The largest positive skin tests occurred with the raw venom, stinger, and whole-body extracts with lesser reactions to the boiled venom and stinger extracts. They concluded that the patient was likely allergic to a protein intrinsic to the bee contained in the venom, stinger, and body. Based on this conclusion, they attempted to desensitize their patient using a combined extract of bee stinger protein mixed with whole-body protein. Beginning with a 1:10,000 dilution, they administered increasing doses every 2 or 3 days until reaching 0.8 cm^3 of a 1:100 dilution. A number of field stings resulted in "no ill effects" up to a year after the therapy.

In 1937, at the 21st Annual Session of the American College of Physicians, Benson presented additional findings from ongoing research regarding hymenoptera allergy. He published his revised remarks to a wider audience in the *Archives of Internal Medicine* in 1939 [22]. This work updated the evolving literature from his earlier manuscript and added reports of reactions to other flying hymenoptera including wasps, hornets, and yellow jackets. His 1939 paper described the evaluation and treatment of 11 hymenoptera allergic patients, 10 new subjects in addition to the patient from his earlier publication. The ten new patients were reactive to a variety of flying hymenoptera including bees, hornets, wasp, and yellow jackets. Their reactions included large local swelling, urticaria, and "collapse with unconsciousness." Benson's findings demonstrated consistent positive reactions to scratch testing with venom, stinger, and whole-body extracts in all patients. Furthermore, he believed that a more important immunologic finding was that "venoms caused no stronger reactions in any of these sensitive patients than did the extracts of bees' bodies from which the stings and poison sacs had been removed." He also noted that five of the patients did not have any relevant pollen sensitivities thereby excluding the possibility of pollen as a source of their symptoms. In five of the patients, he performed supplemental intracutaneous skin tests and concluded that they were advantageous because they provided earlier and

more pronounced immediate wheals. In addition, he noted that some of the intracutaneous reactions resulted in "delayed infiltrations that were red, warm, raised and painful and which persisted for days." He observed that the more dramatic delayed reactions corresponded with the severity of the reactions to stings. Important to the peer reviewers of his original manuscript, he was able to demonstrate passive transfer by the Prausnitz-Kustner technique for one of the patients. For six of the patients, he utilized immunotherapy with the combined extract of bee stinger protein mixed with whole-body protein as reported in his original paper. Benson did not believe that sting challenges were ethical, so assessment of the efficacy of his therapy was limited to one patient who experienced a field sting. That patient suffered only an "ordinary small wheal." Ultimately, he concluded that the patients' reactions were due to an allergen "inherent in the insects' bodies" and that the body and the venom alike contained "the identical allergen of the bees' protoplasm." Benson's paper describing the evaluation and treatment of these 11 patients using whole-body extract, only two of whom experienced field stings without reaction, outlined the common and accepted approach to stinging insect allergy for the next 40–50 years.

In 1953, Mueller and Hill published a manuscript "Allergic Reactions to Bee and Wasp Stings" in the Medical Progress section of the *New England Journal of Medicine* [23]. Their review detailed the accepted understanding as well as unknown aspects of stinging insect allergy. They cited Benson in concluding that "although not entirely clear the sensitizing antigen seems to be contained in the entire body of the insect." Regarding cross-reactivity among hymenoptera, they concluded that there were both species-specific and generic-specific antigens but qualified that "there has been no completely convincing study of cross-sensitivity, and the answer to this is certainly undetermined." They raised the uncertainty regarding the duration and efficacy of therapy citing evidence for both "desensitization" resulting in complete and long-lasting protection with as little as one season of inoculations and "hyposensitization" resulting in incomplete and short duration of protection. Regardless, the evidence available was limited to anecdotes and small case series totaling fewer than 20 patients. Despite limited data and uncertainty, in a "Practical Matters" section, they outlined several specific approaches for consideration. They discussed the uncertainty of accurate identification of hymenoptera suggesting children were unlikely to differentiate among any hymenoptera. They discussed that adults might be able to differentiate between bees and wasps but would be unlikely to differentiate among the wasps. They recommended "to use bee extract alone only when the situation is entirely clear and, in all other cases, to use a mixed extract of bee, paper wasp, hornet, and yellow jacket." Interestingly, they did not outline using skin testing for diagnosis or differentiation. Rather, they outlined simply performing one intracutaneous test with a 1:1,000,000 dilution to determine the starting dose of immunotherapy; if the test was negative, then therapy was to begin at that dilution. Inoculations would then be given once or twice weekly in a 31-shot buildup regimen through six serial dilutions starting at 0.05 cm^3 of 1:1,000,000 and ending at 0.4 cm^3 of 1:10. The authors recommended that the "top dosage" be given once per month for 3 years. Finally, they conveniently listed seven manufacturers with their

addresses in locations throughout the United States where commercially available extracts could be ordered by the practicing physician.

In 1956, Loveless and Fackler published their thorough and exacting work on wasp venom allergy [24]. After her initial experience with whole-body extract immunotherapy from 1948–1952, Loveless was interested in limiting the exposure of patients to the multitude of antigens present in a whole-body extract because of concern for sensitizing patients to possibly extraneous antigens [24, 25]. She also hoped to control the volume of extract administered. It has also been reported that she had suspicions that the use of conventional whole-body extracts "had little if any value in immunizing systemically sensitive patients" [25]. As a result, in 1952, she began studying the use of isolated venom for the diagnosis and treatment of wasp allergy leading to her publication with her assistant Fackler in 1956 [24, 25]. They described harvesting the insects via a butterfly net and then anesthetizing them prior to individually dissecting each venom sac [24]. The venom sacs were then refrigerated in glycerin for preservation. Within 2 h of use, the sacs were crushed to obtain the isolated venom. The study included 34 control subjects and 12 stinging insect-allergic patients. The work detailed the use of isolated venoms for skin testing and conjunctival challenge and for the determination of cross-reactivity among yellow jacket, bald-faced hornet, paper wasp, honeybee, and bumblebee venoms. Additionally, their paper examined dose ranging of venom immunotherapy to define both efficacy and toxicity. Loveless and Fackler explored the use of single- and multiple-day preseasonal rush immunotherapy schedules and documented therapeutic efficacy in seven of the patients using combinations of field stings and intentional sting challenges. Their thorough and detailed manuscript was scientifically a significant step forward in many regards. However, practically, it went unregarded and unutilized. The use of 1- and 2-day rush immunotherapy protocols and the employment of sting challenges presented safety concerns to some practitioners. Additionally, the detailed and exacting science was an obstacle to practical use. Mueller and Hills identified the easy commercial availability of whole-body extract from seven different geographically distributed manufacturers [23]. In contrast, there were no commercially available venom products. As a result, Loveless's methods would have required practitioners to annually capture/harvest live insects; anesthetize, surgically dissect, preserve in glycerin, and refrigerate the venom sac; and finally individually prepare the final isolated venom extract within 2 h of use [24]. Only speculation can be applied to wonder if other factors may have been obstacles to pursuing her important findings.

In 1959, Mueller published his additional experiences with stinging insect allergy again in the *New England Journal of Medicine* [26]. He described his experience with 120 patients suffering with stinging insect allergy. In keeping with his earlier work, he provided broad ranging recommendations on skin testing and therapy based upon his review of the literature and his ongoing research [23, 26]. In this update, he reconsidered his initial recommendation that practitioners should attempt to determine the specific insect that caused the sting reaction so that individual hymenoptera-specific therapy could be used [26]. He concluded that the history of stinging circumstances and stinger retention was "helpful in a presumptive

identification" but that "in no sense can it be positive unless the stinging insect is captured and brought in for identification"—an event that had only happened three times in his experience. Regarding skin testing, he noted the work of Loveless but discounted the need to use "venom sac contents" based upon his assessment of the existing literature. He concluded that it was "satisfactorily answered" that the antigen causing reactions is present throughout the insect's body, independent of the venom, but that the venom contains a small amount of this antigen." He summarized that "scratch testing was found to be an unreliable index of degree of skin sensitivity" and that most patients demonstrated cross-species sensitization with intradermal testing. As a result, he concluded that "there is no reliable way of determining" a specific extract for a given patient from the skin testing. However, he did recommend intradermal testing with serial dilutions of whole-body extract in order to determine the safe starting dose of immunotherapy. Given the lack of confidence in the history and lack of clarity from skin testing, he chose a polyvalent extract of "all" four insects (paper wasp, hornet, yellow jacket, and mixed honeybee/bumblebee) in his study of efficacy. Using the results of intradermal skin testing, he chose to start patients on whole-body extract at dilutions from 1:100,000,000 to 1:1000. He used a five-shot increment to work through each dilution with injections occurring once to twice per week until reaching a maintenance dose of 0.2–0.3 mL of a 1:100 dilution. He reported the outcomes of therapy in 76 patients observed in his academic and private practice from 1951–1959. Of the 76 patients, 63 were on maintenance immunotherapy at the time of reporting, and 13 had completed a 3-year course. There were no intentional sting challenges, but 30 patients reported 48 field stings including four of the stings occurring in patients 1–4 years after completing 3 years of therapy. While there were five systemic reactions, only one of the reactions occurred in a patient on maintenance immunotherapy, and none occurred in patients having completed therapy. Interestingly, his recommendations added new instructions for patients and physicians to provide sublingual isoproterenol for use in the event of field stings. He also identified approaches to schedules and situations regarding maintenance immunotherapy that varied from the uniform monthly maintenance injections. He allowed for consideration of maintenance injections every 4 weeks in the insect season and every 6 weeks throughout the winter. He noted that several patients who had gone as long as 3 months without treatment were able to resume therapy without reducing the dose. His review and recommendations served as a practical update that discounted the value of history and diagnostic skin testing, recommended polyvalent whole-body extract to all the available flying hymenoptera and likely served as reassurance for any that doubted the efficacy of whole-body extract immunotherapy.

In 1961, Shaffer published a review "Stinging Insects—A Threat to Life" in the *Journal of the American Medical Association* [27]. The review identified that a "difference of opinion among allergists" existed "as to whether venom sac contents alone or extract from the whole body of the insect should be used for diagnostic tests and treatment." But Shaffer concluded, "it is generally accepted that the insect whole-body extracts are more potent antigenically and are the treatment material of choice." He recommended commercially prepared single-insect

whole-body extract for intracutaneous testing to establish the initial dose of immunotherapy followed by the use of multiple-insect mixture of whole-body extract in much the same manner as Mueller. Shaffer's review made no reference to Loveless' work. He added a review of three additional studies reporting the retrospective observational experience of efficacy with whole-body extracts [28–30]. These studies added 78 patients to the existing 79 patients from Mueller. The were 37 additional field stings in these 78 patients, and there were not any additional systemic reactions in the whole-body extract-treated patients. In one of those papers by Thomas, he stated "I am convinced from the above observation that all patients who have even severe local reactions as well as systemic reactions, should be considered for hyposensitization." Curiously, the "above observation" did not include any reference to any patients with only "severe local reactions" [30]. In his discussion, Shaffer mentioned Thomas' recommendation [27]. Without further comment or evidence, one of Shaffer's concluding statements was "those persons who have experienced severe local and/or generalized allergic reactions from previous stings should, however, receive desensitization therapy…" Thus, with seemingly little scientific merit, the message to consider immunotherapy for large local reactions was delivered to a wide audience of medical practitioners without qualification. Also of interest from his paper, Shaffer's review expanded on Mueller's treatment plan and provisions for field stings. He recommended providing an emergency kit that included a tourniquet (to apply between the sting site and the body and tighten to reduce venous return but not prevent arterial pulsation), tweezers (for removing retained stingers with care to avoid squeezing the venom into the wound), 10 mg sublingual isoproterenol tablets, an epinephrine aerosol-metered dose inhaler, and instructions.

In a 1965 publication in the *Journal of the American Medical Association*, the Insect Allergy Committee of the American Academy of Allergy detailed the results of several years of accumulated survey data maintained in a registry of over 3000 patient replies [31]. The report included a group of 647 patients who were undergoing immunotherapy and who had been re-stung. From the group, 580 (89.6%) described improvement compared to their pretreatment reaction. In contrast, in a group of 763 patients who were untreated and who had been re-stung, 492 (65%) reported progressively worsening reactions. The physicians of 217 of the patients receiving immunotherapy were contacted and confirmed that all but nine of the patients were receiving multiple-insect whole-body extract, adding that 148 of the extracts were from the same commercial source. While on the surface this data seemed reassuring, the reporting of a subpopulation from the registry revealed possible limitations of recall, of retrospective patient survey data, and of incomplete understanding of the natural history of stinging insect allergy. The subgroup was a fixed group of registrants actively and quasi-prospectively followed by the committee. The group was polled regarding their current experiences but limited to answer only about experiences since entering the registry. This population included 232 patients undergoing immunotherapy who were re-stung. Among this treated group, 88.9% reported improvement, 6.5% were unchanged, and 4.6% were worse. In a group of 102 patients not undergoing therapy who were re-stung, 39% reported

improvement, 34% were unchanged, and 26% reported worsening. While the results remained favorable for the treatment group, the differences were narrower and demonstrated the importance of understanding the natural history of stinging insect allergy. The insight into the natural history of stinging insect allergy as well as the improved study design applied to the subgroup foreshadowed upcoming developments in stinging insect allergy.

The Insect Allergy Committee report also included other recommendations beyond their conclusion that "hyposensitization is clearly of benefit" [31]. They discussed that there was "a growing tendency among our committee members to consider (immunotherapy) also for the persons who show unusually large local reactions in the absence of any systemic symptoms." However, their final recommendations were for individualized decisions in patients with large local reactions. They also summarized groups of patients with possible treatment failure or relapse of systemic reactions after discontinuing immunotherapy for varied time periods. In response, they advocated advancing the maintenance dose to 0.1–0.3 cm^3 of 1:10 of commercially available multiple-insect whole-body extract if tolerated. Additionally, they cautioned that lengthening intervals beyond 4 weeks was not recommended and that "hyposensitization should be considered for an indefinite period until further knowledge is accumulated."

Also in 1965, two articles were published with study designs including larger populations and robust controls. The results of these studies conclusively discounted the validity and applicability of skin testing with whole-body extracts [32, 33]. Schwartz examined the results of intradermal skin testing with three different dilutions of a commercially available multiple-insect whole-body extract containing a mixture of bee, wasp, and yellow jackets [32]. He performed the tests in three groups: an insect allergic group, an atopic group, and a nonatopic group. There were no significant differences in the skin tests among the groups. He concluded that "skin tests cannot be effectively used in the diagnosis of insect-sting hypersensitivity." Meanwhile, Bernton and Brown prepared their own whole-body honeybee extract and applied a single 60 PNU intradermal test and demonstrated that 37.5% of a normal male prison population were positive [33]. They went on to perform intradermal skin testing with a commercial extract at two different dilutions and found that 48% of a normal control population had positive skin tests.

In 1970, Brown and Bernton presented a detailed analysis of 400 patients enrolled in the survey registry of the Insect Allergy Committee of the American Academy of Allergy [34]. This same registry was the source of the 1965 report authored by the Committee itself [31]. In this detailed analysis, Brown and Bernton found that 100/108 (92%) of patients treated with whole-body extract did not experience anaphylaxis after being re-stung. However, their careful analysis also revealed that 39/42 (93%) of patients not treated with immunotherapy did not experience anaphylaxis after being re-stung. Curiously, this seemingly important comparative aspect in the control population was not referenced in their final comments where they simply stated that "hyposensitization … was effective in 100 (92%) of the 108 patients." Notably, Loveless was cited in the bibliography, and a brief mention was made that treatment with "fresh venom" was an option. Also of interest regarding

the treatment of field stings, they mentioned that their survey revealed "many of the patients had been taught to inject themselves" with epinephrine using a Tubex system or preloaded syringes.

Increasing Uncertainty Regarding Whole-Body Extract

In 1970, some 40 years after the promise held forth by the work of Benson and Semenov, Reisman published a brief editorial in the *Journal of Allergy* eloquently summarizing the uncertainty regarding the pathophysiology, natural history, diagnostic testing, and treatment for stinging insect allergy [35]. The upcoming decade would provide great insights into the answers for many of the outlined questions and would significantly change the direction of the diagnostic and therapeutic approach to stinging insect allergy.

In 1973, Tornsey published a report of eight cases of fatal reactions to hymenoptera stings in patients treated with mixed-insect whole-body insect immunotherapy [36]. Four of the eight patients had fatal reactions while on maintenance immunotherapy within 1–2 weeks of their last injection. Three other patients had reactions from 3 to 7 months after their last injections, and one patient was having reactions several years after her last injection. Tornsey concluded that the "disturbing" findings brought attention to the "paucity of accurate knowledge" concerning the pathophysiology, natural history, diagnosis, and treatment of stinging insect allergy.

The Purified Venom Era

In 1974, two papers represented an important turning point for stinging insect allergy and heralded the resurgence of isolated venoms for diagnosis and treatment [37, 38]. In March 1974, a group from of Johns Hopkins publish one of several important advances to come over many years. Sobotka and colleagues published their work showing that pure venom testing via basophil histamine release was a reliable method to diagnose stinging insect allergy [37]. They additionally confirmed the important role of reagin or IgE as a pathophysiologic mechanism. Pure venoms led to significant histamine release in 13 of 16 patients with a history of hymenoptera allergy, while none of the 12 controls exhibited significant release. In May 1974, Lichtenstein, Valentine, and Sobotka published a case report in the *New England Journal of Medicine* describing successful honeybee venom immunotherapy in a child who had failed immunotherapy with mixed-insect whole-body extract [38]. Harkening back to Loveless, a 1-day rush protocol was attempted but without success. After completing an accelerated conventional schedule, the patient underwent an intentional sting challenge that resulted in only a local reaction. The maintenance dose of 100 μg of purified venom was chosen somewhat arbitrarily to approximate the amount of venom in two field stings from a

honeybee. These publications revived Loveless' work and led to rapid investigation of the use of isolated venoms for diagnosis and treatment, as well as the development of commercial sources for the venoms.

The latter half of the decade saw important and rapid advances in stinging insect allergy from the group at Johns Hopkins. In 1976, Hunt and colleagues published their work demonstrating the use of single venoms for skin testing for diagnosis [39]. They began with scratch testing followed by intradermal injections of 0.05 mL in tenfold serial dilutions from 1 ng/mL to 1 μg/mL. All 30 insect-allergic patients tested positive to at least one of five venoms (honeybee, yellow jacket, yellow hornet, white-faced hornet, and wasp), and only one of 30 control patients tested positive. Using higher concentrations of 10 μg/mL and 100 μg/mL led to increasing numbers of positive results in the control population. Diagnostic possibilities were expanded with the development of the first pure venom RAST by the Johns Hopkins group in 1978 [40].

In July 1978, in the *New England Journal of Medicine*, Hunt et al. published a landmark study comparing venom and whole-body extract immunotherapy in a prospective, single-blind, placebo-controlled trial [41]. Fifty-nine patients with suggestive histories and positive venom skin tests were enrolled into three treatment groups: 19 in the venom treatment group, 20 in the whole-body extract treatment, group, and 20 in the placebo group with histamine solution injections. Venom therapy was begun with a 0.1 μg injection using a single-day cluster schedule to reach 10 μg followed by weekly doubling dose injections to reach a maintenance dose of 100 μg. Sting challenges were performed and demonstrated that 1/18 (5%) of venom patients experienced a systemic reaction, while significantly ($p < 0.01$) more patients experienced systemic reactions from both the whole-body extract-treated group, 7/11 (64%), and the placebo-treated group, 7/12 (58%). After the second severe reaction in the non-venom-treated patients, sting challenges were halted, and patients from the placebo and whole-body extract groups were offered venom immunotherapy. All of the additional 40 patients reached maintenance levels of venom immunotherapy. An additional 31 patients were re-stung, and none experienced systemic reactions. In 1979, purified venom extracts became commercially available thus allowing the practical widespread use of this important therapy.

By the close of the 1970s, further advancements in stinging insect allergy were underway. In the decades since, reliable skin testing methods, a serologic method to assess venom-specific IgE, and the reintroduction of deliberate sting challenges have improved the diagnosis and monitoring of stinging insect allergy as well as allowed for improved definition of the natural history of stinging insect allergy. Work continues to advance the understanding of stinging insect allergy in areas such as the safety and efficacy of variable doses, schedules, and durations of immunotherapy, association of venom allergy with mast cell disorders, and improved sensitivity in diagnostic methods. Lessons of the past have taught us to increasingly move away from accepting anecdote, small case series, and retrospective review and move toward applying greater standards of evidence-based medicine to scrutinize these advances. Future chapters of this book will explore the development and scrutiny of these advances.

A Brief Look at the Other Stinging Insects: The Ants

Worldwide there are several species of stinging ants [42]. However, there are very few ants that cause anaphylaxis and human morbidity and mortality with sufficient frequency to generate a sufficient body of medical research beyond anecdote and small case series. In the United States, the dominant species of stinging ant is *Solenopsis invicta* [42, 43]. Imported fire ants were brought to the United States from South America through seaports in the south in the early part of the twentieth century. The aggressive *Solenopsis invicta* became the dominant species spreading throughout much of the southern United States over the course of the twentieth century [44, 45]. A small number of patients experiencing systemic reactions were reported in the 1950s [28, 30, 46]. However, it was not until the 1970s that larger case series of patients experiencing anaphylaxis were reported [46–48]. Since the 1970s, methods of skin testing and immunotherapy with whole-body extracts have been well described. In 1997, Freeman demonstrated the growing importance of systemic reactions to imported fire ants [49]. He reported that in fire ant endemic areas, they are the most frequent cause of hymenoptera hypersensitivity. The evolution of the body of evidence to define the natural history, reliable testing, and parameters for efficacious immunotherapy remains active today.

In Australia, the dominant species of stinging ant is *Myrmecia pilosula*, the jack jumper ant [50, 51]. Although the geographic distribution and the number of patients affected by the jack jumper are not on the scale of the imported fire ant in the United States, it is regionally important in Australia [50]. Much like the imported fire ant, in endemic areas, the jack jumper ant results in double the emergency department visits for anaphylaxis compared with honeybees [51]. Like the imported fire ant, the development of evidence to define the natural history, reliable testing methods, and parameters for efficacious immunotherapy is an active area of discovery for the jack jumper ant.

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Chapter 2
Entomological Aspects of Insect Sting Allergy

Robert S. Jacobson

To provide a biological framework for understanding stinging insects, a basic taxonomic review is warranted. All stinging insects belong to the order Hymenoptera, which translates as "membrane winged." This order is divided into two suborders: the Symphyta (sawflies and horntails) whose members are considered more primitive and are completely incapable of stinging, and the Apocrita which includes the more advanced taxa with many groups capable of stinging. The Apocrita is comprised of two groups, the first being the Terebrantes (previously known as Parasitica) many of which, as their name suggests, typically parasitize other insects and are of considerable economic benefit. Only a few taxa in the Terebrantes are capable of a weak poking of their ovipositor. The remaining group is the Aculeata which include all the true stinging forms—the ants, bees, and "true" wasps. This is the group that will be addressed in this chapter, even though some members have secondarily reduced stings rendering them incapable of stinging (e.g., some ants, as pointed out below). Figure 2.1 illustrates the taxonomic placement of the families having the greatest allergenic importance.

Both social and solitary members of the Aculeata sting as a means of individual defense, such as when bodily movement is restricted by being held or squeezed, but social species also "volunteer" to sting when their colonies are disturbed or damaged. Although field experience will generally teach one as to what circumstances will elicit stinging and what behavioral signs indicate that stinging is imminent, most patients are not in a position (or desire the opportunity!) to gain such knowledge firsthand (though a traumatic incident may have led to their becoming venom allergy patients in the first place and stimulate a vicarious interest in learning more about the offending insect). However, there are ways to minimize the likelihood of stings which will be addressed for the respective taxa discussed below.

R.S. Jacobson, M.S.
Senior Technical Specialist (Retired), Greer Laboratories Inc.,
Lenoir, North Carolina 28645, USA
e-mail: jacobsonbob@yahoo.com

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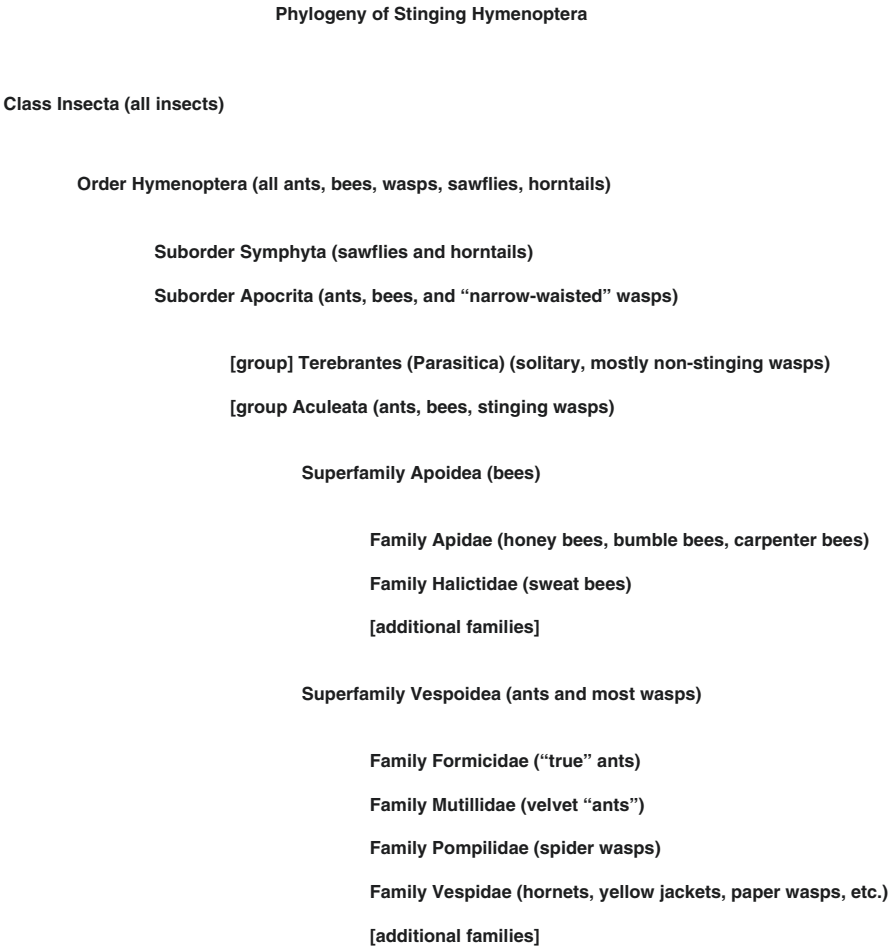


Fig. 2.1 Phylogeny of stinging Hymenoptera

Only the female insects are capable of stinging, and their sting apparatus has been described in detail [1]. Male genitalia possess "claspers" which are used in mating and are harmless although the insects typically go through threatening motions if held and will often protrude their genitalia. In the case of social species, females may be either workers or queens, and for many taxa the queens, which are capable of stinging, generally use their stings only for individual defense. In exceptional situations "foundresses," initiating a new colony, will attack and sting if their small nests are disturbed. Of course the queen honey bee is well known for her intolerance of rival queens, resorting to stinging such rivals, but otherwise will rarely sting even if held.

One of the most important taxonomic families within the Aculeata is the family Formicidae which comprises the ants. Within the ants are about 16 recognized

subfamilies, not all of which are capable of stinging. For example, the subfamily Formicinae, also known as the formicine ants, defend by biting and spraying formic acid at their enemies. Two well-known members of this subfamily are the large and conspicuous carpenter ants (genus *Camponotus*) that usually build their nests in wood, and the somewhat smaller mound-building ants of the genus *Formica*. The formic acid-releasing abilities of these ants can be demonstrated easily by grabbing a worker ant and smelling the acid exuded from the ant's abdomen (a procedure not recommended for ant-allergic individuals). The genera responsible for most medically important stings in the United States are not in the Formicinae but within the subfamilies Myrmicinae and Ponerinae. The Myrmicinae includes the most important stinging genus—*Solenopsis* or fire ants.

Ants are native to all continents except Antarctica, and are also found on most large islands. All ants are social, meaning that they live in colonies having one or more queens (or in a few cases only specialized workers that are physiologically and behaviorally like queens). The workers are sexually underdeveloped females that normally do not lay eggs but are responsible for the other tasks within the nest, such as obtaining food, nest building, feeding the larvae, and defending the nest. Queens and male ants (the latter having been reared from haploid eggs due to parthenogenesis, as is normally the case with all the Hymenoptera) possess wings, while workers are always wingless. In contrast, the queens have wings attached in such a way that they are easily removed. Ant colonies generally reproduce by "swarming" in that when the season and weather conditions are suitable, males and new queens of a given species rush forth from the colony and fly away. Other colonies of the same species in an area also swarm, facilitating cross-mating. After the males have mated, their life span is limited, and they often fall prey to other insects, spiders, etc. The newly mated queens land, remove their wings, and then find a small cavity in which to initiate a new colony. These queens of most species do not forage for food—they use the resources stored in their wing muscles to produce and regurgitate food for the larvae that hatch from the first eggs they lay (all the species within the order Hymenoptera have four life stages: egg, a grub-like larva, a mummy-like pupa, and finally the adult stage). Eventually a small group of undersized workers appears, and they forage for food to nourish the queen and larvae, such food often being living or dead insects, depending upon the species of ant. Thus, the colony grows as more workers are produced, and eventually males and new queens are reared. Ant colonies can live for several years, so the same colony can release swarms several times over the course of its duration. Some species may have less than a hundred adults in a colony, while others may have well over 100,000 [2].

Although there are several species of fire ants, the two most important species in the United States are introductions from South America, which include the red imported fire ant (*Solenopsis invicta* Buren) (Fig. 2.2) and the black imported fire ant (*Solenopsis richteri* Forel), sometimes abbreviated as RIFA and BIFA, respectively. (Zoological names are composed of a genus and a species (both italicized), plus the name(s) of one or more authors who originally described the species, the latter not being italicized. The generic name may be abbreviated after the first usage for a given species. If a taxonomic revision has placed the species into another



Fig. 2.2 *Solenopsis invicta* (imported fire ant) workers. https://upload.wikimedia.org/wikipedia/commons/9/94/Fire_ants_01.jpg ([Origin: Stephen Ausmus; Image Number K11622-1; http://www.ars.usda.gov/is/graphics/photos/dec04/k11622-1.htm Image K11622-1.jpg "Fire ants"] 1058 × 1890 pixels. Photo in public domain per ARS-USDA website)

genus since the original description, the author's name is placed within parentheses; otherwise, no parentheses are used. If a subspecies is being cited, the author of the subspecies is used rather than the name of the original describer of the species.) In spite of their names, these ants are both brown, though *S. richteri* is much darker, and typically has a lighter area on the abdomen, while *S. invicta* is more uniformly chestnut brown. Although the black species was the first of the two to be introduced into the United States via Mobile, AL in 1918 from South America, it has been largely displaced by the red species (introduced in the early 1930s) [3], causing its range to essentially be restricted to eastern Mississippi and western Alabama. The two species do not interbreed in their native continent, but hybridization takes place in their new home, with *S. richteri* losing ground to *S. invicta* both literally (geographically) and genetically (pure *S. richteri* is becoming scarce), and *S. invicta* remains the dominant species from Central Texas eastward to and throughout Florida, and northward through eastern North Carolina, with outlying introductions. The species is also established in Puerto Rico [4, 5]. There is evidence that the hybrid ants have greater physiological resistance to freezing temperatures [6], which

may allow their range to extend northward, including the southern tiers of counties in Tennessee. These are small ants, with workers being from about 3–5 mm in length, and having clubbed antennae. Both species of *Solenopsis* and their hybrids build mounds sometimes a foot or more in height, and both the mounds and the ground under them contain numerous tunnels and chambers essential for providing a suitable temperature for the brood being reared in the nest. Additional background information about the biology of imported fire ants can be found in Rhoades 1977 [7]. Although tunnels radiate from each mound underneath the soil surface, the mounds themselves have no obvious entrances; however, any disturbance to a mound, especially if the surface is broken, results in a frenzy of workers that rush out to attack and sting the intruder. Envenomization is achieved by each ant fastening to the enemy's body with its mandibles and then stinging one or more times, sometimes making a circle of wounds. If sufficient venom is injected, a sterile pimple-like pustule will form within a day [8]. One can pick up individual fire ant workers between the finger tips without being stung because the stinging apparatus is so small that the ant must brace itself with its mandibles and position its sting to be successful (again, a procedure not recommended for those who are hypersensitive). Most other stinging insects cannot be held safely in this manner.

The native fire ant species—the southern fire ant *S. xyloni* McCook, the tropical fire ant *S. geminata* (Fabricius), which has both small and large workers, with the latter having oversized heads, and the tiny light-colored desert fire ant *S. aurea* Wheeler—are occasionally responsible for anaphylactic reactions [9, 10]. Their colonies are smaller than those of the imported species, and they are generally less aggressive. Where the imported species overlap their ranges, the native species have become less abundant [10]. Stings from fire ants can generally be prevented by learning to recognize their mounds and avoiding them; this is addressed in greater detail below.

In addition to fire ants, two other genera of ants are of some importance in the United States due to their stings: the harvester ants (*Pogonomyrmex*) (Fig. 2.3), and the imported Asian needle ant *Brachyponera chinensis* (Emery) (Fig. 2.4) previously known as *Pachycondyla chinensis* (Emery).

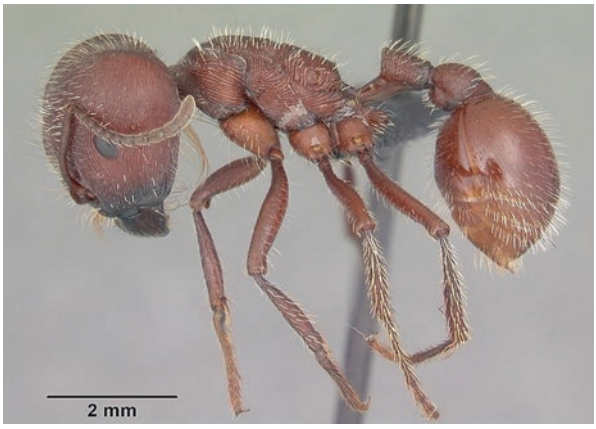


Fig. 2.3 *Pogonomyrmex* (harvester ant) worker. https://upload.wikimedia.org/wikipedia/commons/6/6a/Pogonomyrmex_badius_casent0103057_profile_1.jpg ([Photographer: April Nobile/from AntWeb.org CC-BY-SA-3.0 CASENT0103057] 1037 × 808 pixels. License: Attribution-Share Alike Creative Commons License)



Fig. 2.4 *Brachyponera chinensis* (Asian needle ant) worker. https://upload.wikimedia.org/wikipedia/commons/a/a0/Brachyponera_chinensis_casent0104738_profile_1.jpg ([Photographer: April Nobile/from AntWeb.org CC-BY-SA-3.0 CASENT0104738] 1037 × 808 pixels. License: Attribution-Share Alike Creative Commons License)

Harvester ants are found in the drier areas of the western United States and adjacent Canada, with one species (*P. badius* (Latreille), the Florida harvester ant) extending across the Gulf States to the East Coast. Other species are found in Mexico and Central and South America. They are relatively large ants, and at least in the case of the Florida harvester ant, the largest workers have particularly enlarged heads to house the muscles and mandibles needed for biting through the seeds the ants gather and store in their nests. Colonies are easily recognized by their large mounds (rather flat in some species) that have a distinct bare area around them caused by the ants' chewing off the vegetation; these mounds may exceed 4 ft in diameter. Although the species vary as to their defensiveness, their venoms are the most toxic insect venoms currently known (based upon LD50 in mice) [11, 12] and in humans tend to cause a throbbing pain that spreads to the lymph nodes. Anaphylaxis caused by harvester ant stings has been reported by Klotz et al. [9]. As with fire ants, preventing stings from harvester ants is best achieved by avoiding their nests.

The Asian needle ant (*B. chinensis*), as the name suggests, is an introduction from eastern Asia (from Japan and Korea to Vietnam, the Philippines, and Thailand) initially collected on the East Coast of the United States in 1932, in the Norfolk, VA, area and was soon found to be well established in eastern North Carolina, eventually being reported as far west as Tennessee. This species is approximately the same size as a larger fire ant worker but has a more "cylindrical" shape (particularly in the gaster or continuous portion of the abdomen) and one "hump" or segment in the petiole (in contrast to the two found in fire ants and harvester ants) connecting the thorax with the gaster typical of the ponerine subfamily. They are omnivores though apparently preferring termites as food. Needle ants are not aggressive but will sting if squeezed against the flesh. A description of the local effects of their sting from the "School of Ants" website mentions an immediate slight burning sensation followed by spreading of the painful area to about an inch diameter and remaining sensitive for about 2 weeks [13]—however, published reports indicate variable local effects [14]. Anaphylaxis has been documented in Korea (e.g., Klotz et al. [9]) and in the United States [15]. Needle ant nests are small and inconspicuous. Stings are best

avoided by inspecting outdoor objects before picking them up and by wearing gloves while gardening or otherwise working with soil and objects in contact with soil.

Other notorious ants of the world include the "bullet" ants (*Paraponera clavata* (Fabricius)—a ponerine species) of South America and southern Central America, known for their extremely painful stings, and the "bull" ants of Australia (various species of *Myrmecia*, particularly the aggressive *M. pilosula* F. Smith commonly known as the "jack jumper" ant); the stings of the latter genus are responsible for anaphylactic reactions in addition to pain. (The genus *Myrmecia* belongs to the subfamily Myrmeciinae, which is different from the subfamily Myrmicinae in spite of the similar name.)

Vespidae

The members of most families of aculeate wasps are solitary. The family Vespidae is an exception that contains both truly social and solitary species. Solitary species do not live in colonies or have a worker caste, but typically a single female mates, seeks appropriate food such as prey that is paralyzed by a sting, and stocks her small nest with this prey and then abandons the nest after laying one or more eggs. Regardless of whether they are solitary or social, most aculeate wasps are capable of stinging, with some of solitary members known for their painful stings (e.g., the wingless "velvet ants" or "cow killers" of the family Mutillidae sometimes seen crawling on the ground, especially in sandy areas, and the large tarantula hawks of the genus *Pepsis* (in the family Pompilidae commonly seen in the southwestern deserts and whose sting earns the highest rating of 4 on the Schmidt sting pain index)) [14]. However, the real culprits causing mass stinging or anaphylactic reactions in this family are not the solitary species, but the social species.

Within the family Vespidae are several subfamilies—a few being solitary while others are social. The largest colonies in the temperate regions of the Northern Hemisphere are built by members of the all social subfamily Vespinae which includes the genera *Vespula*, *Dolichovespula*, *Vespa*, and the nocturnal genus *Provespa*. In the United States and Canada, the first two genera are responsible for most stings from this subfamily. One of the many species of *Vespa* has been introduced to North America from Europe, while the three species of *Provespa* are strictly from tropical and subtropical areas of southern and southeastern Asia with none having become established elsewhere; therefore, the latter genus will not be addressed further here.

The life cycles of the various vespine species are similar. An individual overwintered queen becomes active in the spring and builds a small nest of paper mainly from chewed wood pulp containing perhaps a dozen cells, depending upon the species and circumstances. An egg is laid in each cell, and after each hatches, the queen collects animal protein, usually by predation, and feeds each larva. The queen also collects wood pulp to enlarge the nest, including lengthening the cells to accommodate the growing larvae, and protects the nest. After the larvae mature, become

pupae, and emerge as adults, the newly emerged workers gradually replace all the functions the queen has been performing except egg laying. The nest continues to grow, and a large population of workers is reared. Usually around late summer or autumn, stingless males and new queens are reared. (Some researchers prefer to call the young females "gynes"—reserving the word "queen" until the female insect initiates a new colony.) At this time, males and new queens mate (generally from different colonies), the old queen dies, the workers gradually die off, the males leave and succumb to cold weather or predators, and the newly mated queens seek a location to hibernate. The cycle is repeated the following spring when the new queens become active again.

The genus *Vespula* contains two distinct groups of species, with the appropriate taxonomic placement of a smaller third group still subject to differences of opinion. The first group, often called the *V. vulgaris* group but sometimes referred to by European researchers as *Paravespula* [16], include species that produce large colonies whose workers fulfill most of the their brood's need for protein by scavenging which, in addition to their tendency to be abundant, leads to frequent encounters with people and thus stinging incidents. Some members of this group have been successful in colonizing new areas; for example, *V. pensylvanica* (de Saussure) has become established in Hawaii, *V. vulgaris* (Linnaeus) is now abundant in New Zealand, and *V. germanica* (Fabricius) has colonized much of the United States in addition to southern South America, South Africa, and many other regions. Members of this group native to North America include the widespread and common eastern *V. maculifrons* (du Buysson), the somewhat more restricted eastern *flavopilosa* Jacobson, and the widespread *alascensis* (Packard) restricted to cooler regions, the last species having been recently recognized as distinct from the true *V. vulgaris* native to the Old World [17]. These three native species along with *V. vulgaris* (Fig. 2.5), which resemble each other rather closely, build nests frequently containing a couple thousand adults and use decayed wood such as a rotting stump as their



Fig. 2.5 *Vespula vulgaris* worker. https://upload.wikimedia.org/wikipedia/commons/9/9b/Flying_Vespula_vulgaris.jpg ([Photographer: Soebe] 715 × 635 pixels. License: GNU Free Documentation License)



Fig. 2.6 Nest of *Vespula vulgaris*. https://upload.wikimedia.org/wikipedia/commons/e/e6/Vespula_germanica_nest_-_Zoo_Frankfurt.jpg ([Original photo misidentified as Vespula germanica nest; photographer JuTa] 1976 × 1680 pixels. License: Creative Commons Attribution-Share Alike 3.0 Unported)

pulp source. The resulting nest is comprised of brittle paper the color of sawdust (Fig. 2.6). Nests are typically, but not always, constructed underground. Each nest begins with an individual overwintered queen selecting a location, such as a rodent burrow or a space remaining from a decayed tree root, and building a golf ball-sized nest containing a dozen or so cells. She lays an egg in each cell and rears the first brood of workers that, in turn, take over most activities of collecting pulp and expanding the nest, procuring food, and defending the nest. This allows the queen to focus on egg laying. The nest of a mature colony often exceeds the size of a volleyball and contains five to perhaps a dozen combs. Cells used for rearing queens, built later in the season, are much larger than those that produce workers and males, with entire combs in the lower portion of the nest dedicated to rearing new queens. Combs of vespine wasps are oriented horizontally within the nest, and the single layer of cells in each comb opens downward. The oldest comb, with a distinct center core representing the queen mother's original endeavors, is at the top, and each successive comb is built underneath it. The combs are used strictly for rearing brood through three stages (i.e., egg, larva, and pupa), and the immature insects are oriented with their heads downward at the opening of the cells. At the end of the growth phase, each larva spins a cocoon inside the cell, resulting in a silken "cap" at the bottom.

Within both *Vespula* and *Dolichovespula* are a few species that do not establish their own nests, but whose queens invade incipient colonies of other species and eventually take over egg-laying functions. The workers of the host species then rear the new queens and males of the parasitic invaders as the latter do not produce workers. These parasitic or inquiline species present no stinging threat unless their queens are held, although the workers of the host species will defend the nests if disturbed. Thus, the parasitic species are not discussed further.

Vespula pensylvanica (de Saussure) (Fig. 2.7) and *Vespula germanica* (Fabricius), the former being native (to the *western* half of the United States, in spite of its name which was misspelled in the original description of the species therefore requiring



Fig. 2.7 *Vespula pensylvanica* queen. https://upload.wikimedia.org/wikipedia/commons/4/40/Vespula_pensylvanica-Queen-1.jpg (Photographer Eugene Zelenko 1100 × 1100 pixels. License: GNU Free Documentation License)

perpetuation of this misspelling!) and the latter having been introduced into North America from Europe and now widespread in most of the United States and southern Canada, use sound wood for their nests resulting in a flexible gray paper nest.

The second group, usually called the *V. rufa* (Linnaeus) group because of this widespread Old World species of the group having been described first, typically develops smaller colonies built of gray paper, and the workers obtain most of their protein by predation on other insects; for these reasons the members of this group are considerably less important as stinging hazards. People who work in forests or other rural areas, particularly in cooler climates, are more likely to encounter nests made by this group. Native American members of this group include the eastern *V. vidua* (de Saussure), the western largely yellow *V. atropilosa* (Sladen), and the somewhat smaller often "polka-dotted" *V. acadica* (Sladen) typically found in cooler regions. The white-banded "black jacket" *V. consobrina* (de Saussure) and the rare northern red-spotted and white-banded *V. intermedia* (du Buysson) complete this group.

Vespula squamosa (Drury) (Fig. 2.8) and *V. sulphurea* (de Saussure) are present in the southeastern third of the United States and in California and adjacent states, respectively. They are strictly an American group having no close relatives in the Old World. The former is especially widespread and abundant, and both its aggressive defense of its colonies and its tendency to scavenge make it a risk for stinging attacks. Its colonies occasionally overwinter successfully in the southern parts of its range, thus forming huge colonies whose nests may surround a tree trunk or fill a large portion of an abandoned automobile or shed; such colonies present a severe stinging hazard to unaware passersby. Nests made by this group are typically gray, but the former may have areas of sawdust-colored paper in its nests as evidence of having "usurped" small colonies of members of the *V. vulgaris* group.

The name *Vespula* is a diminutive of *Vespa*; thus the name of the former means "little hornets" although they are not true hornets, as discussed below. The various species of *Vespula* vary somewhat in size, but in general workers range from about



Fig. 2.8 *Vespula squamosa* worker. https://upload.wikimedia.org/wikipedia/commons/2/2f/Southern_Yellowjacket_%28Vespula_squamosa%29_%287225863346%29.jpg (Photographer: Bob Peterson 1024 × 1024 pixels. License: Creative Commons Attribution-Share Alike 2.0 Generic)

10–15 mm in length, while queens may be 20–25 mm, depending upon the species. Males are slightly longer than workers but usually not much broader.

The genus *Dolichovespula* (meaning "long [faced] little hornets") includes a few species that are popularly called "hornets" in North America—in particular, the largest species, *D. maculata* (Linnaeus) (Fig. 2.9), is known as the bald-faced hornet. This is somewhat of a misnomer because the true hornets belong to the genus *Vespa* (of which one species is established in the United States and is discussed below). However, the bald-faced hornet is the largest native vespine in the Western Hemisphere, and its size is within the range of some of the smaller true hornets of the Old World. Its large and conspicuous gray aerial nests (Fig. 2.10) are also reminiscent of those made by some species of *Vespa*. Thus, the bald-faced hornet can be thought of as being an oversized, black-and-white aerial species of yellow jacket.

The bald-faced hornet is found throughout the continental United States, much of Canada and some regions of Alaska, but it is absent from the hot deserts and southern areas of the Great Plains. The workers and males are some 20 mm in length with the queens usually exceeding 25 mm. The lengths of individual vespine wasps and many other hymenopterans are variable because the abdomen has a series of overlapping sclerites—that is, individual sections of their chitinous shell—that "telescope," making the length of an insect greater if well fed, full of eggs, or full of fat stored for hibernation. The bald-faced hornet has large white areas of its face, with a few white markings on the thorax and three white bands at the tip of abdomen of workers and queens, four in males. The Old World lacks a species of similar coloration, though a couple closely related species, including the widespread *D. media* (Retzius), have more typical black and yellow "yellow jacket" patterns. The bald-faced hornet is generally unaggressive unless its nest is disturbed or approached too



Fig. 2.9 *Dolichovespula maculata* worker. https://commons.wikimedia.org/wiki/Dolichovespula_maculata#/media/File:Dolichovespula_maculata_fg01.jpg (Photographer: Fritz Geller-Grimm 449 × 599 pixels. License: Creative Commons Attribution-Share Alike 2.5 Generic)



Fig. 2.10 *Dolichovespula maculata* nest. https://upload.wikimedia.org/wikipedia/commons/4/43/Bald-faced_hornet_%28Dolichovespula_maculata%29_nest.JPG (Photographer: "the High Fin Sperm Whale" 1828 × 1824 pixels. License: Creative Commons Attribution-Share Alike 3.0 Unported)

closely; even a slight disturbance will result in a mass of workers leaving the nest and attacking, often flying toward one's face.

The so-called yellow hornet, sometimes called the "aerial yellow jacket," is another widespread member of the genus *Dolichovespula*. This species, *Dolichovespula arenaria* (Fabricius), is also found throughout much of temperate North America but is restricted to cooler climates than the bald-faced hornet; hence, it is not present in the hot deserts, much of the Great Plains, the Gulf States, or at low elevations of the southeastern United States. It looks superficially like some of the members of the genus *Vespula* discussed above, but it has the longer face typical of the genus *Dolichovespula*. There are three other (nonparasitic) species—the similar *D. norvegicoides* (Sladen) and *D. alpicola* Eck, and the white-banded *D. albida* (Sladen)—in this genus found in North America, but these are mostly restricted to northern regions and/or relatively high elevations, thus presenting less danger to human populations. Common names have been contrived for most of the social wasp as well as bumble bees, but many of them are not helpful because they are either not sufficiently descriptive or are more cumbersome than the zoological names themselves, thus being superfluous for specialists and potentially ambiguous for others.

The nests of *Dolichovespula* are made from weathered, but sound, wood such as from fence posts. Consequently, these nests are typically gray in color with strong, flexible paper. Nests are similar to those of *Vespula* except that the envelope is usually thicker (e.g., sometimes over 2 in. in *D. maculata*, with a dozen or more individual sheets of paper) because the nests are usually directly exposed to the elements. Many nests exhibit a form of "scalloping" in the envelope—that is, small sheets of paper fastened on all sides except the bottom. Nests of the bald-faced hornet may reach 2 ft in length and over a foot in diameter, often containing four or five large combs and over 400 adult insects by late summer and early autumn. Nests of the yellow hornet are similar in basic structure but on a smaller scale and with a smoother envelope with minimal or no scalloping. Nests of the latter species in areas of the Pacific Northwest west of the Cascades may approach the size of large nests of *D. maculata*. Because individual insects belonging to *D. arenaria* are smaller than those of *D. maculata*, a large nest of the former is likely to have more combs and individual cells than nests of the latter. The other species of *Dolichovespula* in North America build nests similar to, but generally smaller than, the nests of the yellow hornet to which they are closely related.

The genus *Vespa* includes about two dozen species of true hornets, native only to the Old World. The natural range of one species, *Vespa crabro* Linnaeus (Fig. 2.11), is found throughout most of Europe except the coldest regions, and by 1850 it was established in the United States, expanding from its initial introduction point in the New York City area to cover roughly the eastern third of the United States. In Europe this species is simply called "the hornet" or its equivalent in the various European languages; in North America it is known as the "European hornet" though some people refer to it as the "Japanese hornet" or "giant hornet"—the former two names not technically incorrect because the natural range of the species extends across Europe and Asia to the Pacific Coast, including Japan. The color form established in the United States is the one native to continental Europe and is sometimes



Fig. 2.11 *Vespa crabro* male. https://upload.wikimedia.org/wikipedia/commons/e/eb/Hornisse_5.jpg (Photographer: Flugwapsch 62 5184 × 3456 pixels. License: Creative Commons Attribution-Share Alike 3.0 Unported)

given the subspecific name *germana*. Anaphylaxis has been reported to stings of this species [18]. Most of the other species are native to southern, southeastern, or eastern Asia, along with associated islands as far southward as New Guinea, with the range of one species (*Vespa orientalis* Linnaeus) extending to the Middle East, northern Africa, and some areas of southeastern Europe. Another species, *Vespa velutina* (Lepeletier), has been inadvertently introduced from China into France, becoming naturalized there and in neighboring countries [19].

Nests of the genus *Vespa* may be located underground, inside cavities, or built among branches of a tree or shrub, depending upon the species, with some individual species using various kinds of nesting sites. Their paper is typically of a brown or sawdust color, similar to that of the yellow jackets of the *Vespula vulgaris* group described above. Several species tending to build in cavities typically build only a rudimentary protective envelope. Although the paper produced by *Vespa* is rather brittle, the large size of the insects allows them to produce thicker sheets sufficiently sturdy even when subjected to the weather. Some species are very aggressive in defending their nests, sometimes even if approached, while a few others will even tolerate handling of an inhabited nest.

Vespa crabro resembles a gigantic yellow jacket in body shape and coloration. Workers typically reach some 30 mm in length, being roughly the same size as a queen of *D. maculata*. Queens are somewhat larger. The nest of *V. crabro* can be found in several settings—usually inside a hollow tree or space in a wall but sometimes hanging freely under eaves or at least expanding outside a closed cavity to give the appearance of a freely-hanging nest. Rarely *Vespa crabro* nests are built underground. An extensive envelope is constructed if the nest is exposed; otherwise, the envelope is rudimentary. Cells are large, reflecting the size of the insects themselves, and a nest may contain over a dozen combs, with a few hundred insects present. Although the European hornet is not very aggressive, it will defend its nest if approached too closely or disturbed. It shares a characteristic with *D. maculata* in that it will sometimes spray its venom when attacking; if the venom gets into the intruder's eyes, severe irritation results.

An updated key to the Vespinae is provided in Kimsey and Carpenter [20]. Approximate maps of the geographic distribution of most of the species and extensive biological information is provided by Akre et al. [21].

Another important subfamily of social wasps is the Polistinae, and within this subfamily are several genera, three of which are represented north of the Mexican border: *Polistes*, *Mischocyttarus*, and *Brachygastra*. In the Americas, the Polistinae reach their greatest diversity in tropical regions; this diversity can be appreciated by reviewing the volume by Richards [22]. The taxonomy of the various genera has undergone revision since publication of Richards' work [22].

The genus *Polistes* is well represented over most of the United States and extends into southern Canada. *Polistes* contains most of the so-called paper wasps commonly seen nesting under the eaves of buildings. Other species of *Polistes* occur in most warm regions of the world. Members of this genus build a "bare" nest—that is, a single comb suspended by a petiole that lacks the protective covering or envelope typical of vespine nests. Within this genus there are several subgenera, but the proper division of the genus is still unresolved [23] and beyond the scope of this chapter. Groups of species can be recognized; for example, the widespread species *P. fuscatus* (Fabricius) is found throughout at least the eastern half of the United States and adjacent Canada, and related species including the dark reddish-brown *P. metricus* Say, the yellow *aurifer* Saussure, the small variable *dorsalis* (Fabricius), and the rather large beige and light-brown *apachus* Saussure are widespread within the eastern half, western half, southern third, and the southwest, respectively. All of these species typically build symmetrical nests (assuming a lack of interfering objects) having the supporting petiole in the middle, and males are easily recognized by having yellowish faces even if the background color is dark. *Polistes fuscatus* in cooler regions often retreats rather than attacks if the nest is disturbed, whereas more southern populations and the related species are most apt to sting when disturbed, especially if the weather is warm. Other members of this group include the tannish-gold *P. flavus* Cresson of the southwest, and the two orange-red species *P. rubiginosus* Lepeletier and *carolina* (Linnaeus) of the southeast. A second group contains such species as *P. annularis* (Linnaeus), a large (occasionally exceeding 25 mm in length) dark species sometimes known as the "red wasp," and *P. exclamans* Viereck (a smaller brown- and yellow-banded wasp known in some regions as the "guinea wasp") (Fig. 2.12). These two species inhabit the southeastern third of the United State and are especially thin wasps that build lopsided nests having the petiole near the edge and the comb hanging at an angle such that the cells open somewhat laterally. The former species tends to build its nests near bodies of water, while the latter often uses human dwellings and farm buildings where its service in preying on unwanted caterpillars somewhat offsets the potential stinging hazard. In the southwestern United States are three other species: *P. canadensis* (Linnaeus), *comanchus* Saussure, and *arizonensis* Snelling, the first two resembling *annularis* (although *comanchus* is largely yellow), while the last resembles *exclamans*. An Old World species, *P. dominula* (Christ) (Fig. 2.13), became established in the northeastern United States in the early 1980s and has spread throughout much of the country; its crisp black and yellow coloration sometimes causes it to be



Fig. 2.12 *Polistes exclamans* colony. https://upload.wikimedia.org/wikipedia/commons/5/54/PolistesExclamans_3288.JPG (Photographer: Davefoc 1987 × 2001 pixels. License: Creative Commons Attribution-Share Alike 3.0 Unported)



Fig. 2.13 *Polistes dominula* on nest. https://upload.wikimedia.org/wikipedia/commons/9/97/Wasp_March_2008-3.jpg (Photographer: Alvesgaspar 2301 × 1647 pixels. License: GNU Free Documentation License)

mistaken for a yellow jacket. Its nests, which resemble those of *P. fuscatus* with slightly smaller cells, are frequently built inside structures where the somewhat defensive wasps are easily disturbed. Finally, *P. major* Beauvois, a gold-banded species and its dark variant *castaneicolor*, are found in southern Florida and Arizona, respectively, and represent an additional species group. A few other species are present in the United States but are either uncommon or similar to those already described.

Another group of paper wasps is the large genus *Mischocyttarus*, predominantly found in tropical Latin America, with two species occurring in the United States: *M. mexicanus* Saussure and *M. flavitarsis* Saussure. The former, almost entirely restricted to Florida, is a small wasp some 10 mm in length and rather resembles a tiny *Polistes exclamans* except that the first abdominal segment is elongated, as in all species of *Mischocyttarus*, causing the insects to appear more "wasp-waisted" than the species of *Polistes*. The larger *M. flavitarsis* is about twice the size of

M. mexicanus and is mainly dull yellow with dark markings varying in shade depending on the region (and is suggestive of a small *P. comanchus*!). *M. flavitarsis* has a wide distribution in the western United States. Nests are uncovered single combs, thus similar to those of *Polistes*. The insects are relatively unaggressive. In both *Polistes* and *Mischocyttarus*, the queens are only slightly larger than the workers. In some species of *Polistes*, it may be almost impossible to recognize the queen in the colony by external morphology and size.

The final genus of social wasps to be addressed—*Brachygastra*—is mainly Neotropical and has only one species extending northward into southern Texas, usually south of San Antonio, and has been recorded in Arizona. This species, *B. mellifica* Say, is often called a "honey wasp" because it produces and stores large quantities of a form of honey (something done by very few wasps) within its large round or oval grayish nests that rival those of *D. maculata* in size. However, the nest architecture is very different from that of the Vespinae and the other Polistinae found in the United States—the cells are built on the envelope itself, forming many layers within the nest, which may contain several thousand wasps. The insects themselves are slightly smaller than yellow jacket workers (i.e., typically less than 10 mm in length) and have a different shape—the abdomen in particular has the second segment enlarged and rounded, making it look relatively short, hence the name for the genus ("short gaster" with the latter referring to the visible part of the abdomen). They are mostly black with thin yellow bands on the distal half of the abdomen. *Brachygastra* are normally rather unaggressive, but if the nests are sufficiently disturbed, the wasps will attack *en masse* and sting, with the stings sometimes being left behind as is characteristic of honey bees [24].

Bees

Bees are included in several taxonomic families, but only one family (Apidae) includes the truly social species. Two genera of social bees occur in the United States and Canada: *Apis*, which includes the introduced honey bee *Apis mellifera* Linnaeus, and *Bombus* or bumble bees.

About seven species of honey bees are known (depending upon whose classification is followed, but the most conservative systems admit at least four), and all but one are native to southern, southeastern, and eastern Asia, including associated islands. *Apis mellifera* is native to Europe, Africa, and the western half of Asia but has been introduced to both North and South America, Australia, and virtually every land in which a suitable climate exists. Honey bees have been present in North America nearly four centuries, often appearing west beyond the expanding "front" of settlement by Europeans and their descendants due to swarming [25].

Apis mellifera is made up of several strains, races, and subspecies, but due to crossbreeding and movement by human populations, the phylogeny of the species has become difficult to determine. All *Apis mellifera* forms live in colonies comprised of thousands or tens of thousands of individuals and builds waxen combs suspended vertically with hexagonal cells arranged horizontally on both sides and opening in

opposite directions. These cells are used for the storage of honey and pollen and for the rearing of brood (eggs, larvae, and pupae), with one insect developing in a cell at a time. The adult population includes a laying fertile female or queen, a few hundred males or drones, and the remainder sexually underdeveloped females or workers.

Honey bees produce honey and beeswax, two items of commercial value, but their main economic value is their pollination of commercial food crops. Unlike other social hymenopterans in temperate regions beside ants, their colonies are perennial, allowing them to mobilize a large field force during any time the weather is suitable for flight.

Because of their perennial colonies, the possibility of being stung by honey bees exists virtually any time of the year, even on winter days warm enough for flight. Because of the various strains of bees, differences in condition and health of individual colonies, effects of current weather conditions, and variation in the disposition of different colonies, honey bees range from those whose nests can be approached and even manipulated with minimal defensive behavior to those that attack spontaneously if approached within several tens of feet. Most colonies fall within the middle of these extremes, being unaggressive if the hive is not disturbed and the flight path is not hindered but becoming defensive in proportion to the severity of a disturbance. (The author's own experience with backyard hives included many pleasurable hours spent sitting a couple feet away from a hive watching the activity at the entrance without protective clothing and even included grabbing drones from the entrance while barehanded.)

The danger presented by honey bees has been augmented in the southern third of the country by the presence of the Africanized or so-called "killer" bees. Experiments in Brazil were undertaken during the 1950s to produce a strain of honey bees that would thrive well under tropical conditions, and these included the introduction of bees from Africa, which were of the subspecies *Apis mellifera scutellata* Lepeletier (Fig. 2.14). These Africanized bees appear essentially identical to several of the European strains. Original hopes were that the positive attributes of this African subspecies could be kept while reducing their aggressiveness by crossing with the European strains currently being used. However, a lack of communication among individuals at the facility resulted in the African strains being allowed to escape into



Fig. 2.14 *Apis mellifera scutellata* worker. https://commons.wikimedia.org/wiki/File:Apis_mellifera_scutellata.jpg (Photographer: Jeffrey W. Lotz, Florida Department of Agriculture and Consumer Services, Bugwood.org 3072 × 2077 pixels. License: Creative Commons Attribution-Share Alike 3.0 Unported)

the wild, with resultant spread, eventually covering a substantial area of South America and extending northward through Central America and Mexico to achieve a presence in the United States [26, 27]. Despite the bees being nearly identical to the European strains in appearance (if anything, being slightly smaller) and the quantity and potency of their venom essentially the same, two important differences make them much more dangerous—their nesting habits and their temperament. They tend to swarm frequently and build nests in or under various structures where one would not expect to find honey bees, and very little agitation (such as with one's mere presence within the vicinity of a colony) is necessary to provoke them into frenzied stinging. Southwestern states such as California and Arizona have published descriptive literature providing warnings as well as suggestions as to means of minimizing or escaping attacks, and as other states (Florida) report the occurrence of these bees, additional warnings are being published and posted on the Internet [26–28].

Honey bees have barbs on the lancets of their stings, enabling the entire apparatus to remain in the skin of the victim allowing both the injection of additional venom and the release of a chemical pheromone that attracts additional attacking bees to the victim.

Currently 46 species of bumble bees are recognized in the United States and Canada, though several of these are considered "holarctic" in that they also occur in Europe and/or northern Asia [29]. No exotic species of *Bombus* have become established in North America. Bumble bee bodies are entirely black, while their dense pelage of hairs are of various colors—usually black and yellow—but some species have shades of red, orange, or white, depending upon the species. Queens are typically 25 mm or more in length, while workers and males are about half as long; some species are larger than others. Bumble bees live in annual colonies initiated independently each spring by individual overwintered queens, each of which builds a small nest, provisions it with pollen and honey, and lays a group of eggs eventually developing into workers—that is, their colonies follow the same basic life cycle as those of the Vespinae. Successive clusters of eggs are laid as the older immatures go through the larval and pupal stages of development and finally emerge as adults. Unlike the social wasps and honey bees, bumble bees do not build combs; rather, their nests appear as an untidy cluster of yellowish-brown cocoons, some of which are open to serve as "honey pots." The entire nest is unlikely to exceed the size of a grapefruit, and it may be built in an abandoned rodent burrow, under a clump of grass, inside a birdhouse, or even in abandoned furniture where the interior stuffing has become exposed. Populations may reach or exceed 100 individuals, depending upon the species. Bumble bees are not aggressive away from their nests, resorting to stinging only if squeezed, but defensive behavior in or near a colony varies among species, some of which are extremely persistent in attacking an intruder. Their size and strength often enable them to sting through clothing or force their way under clothing and sting the skin directly. Before flying to attack, bumble bees often vibrate their wings in a threatening manner making a warning noise, so a person may have sufficient opportunity to leave the vicinity of the nest before the bees actually appear. Observation and listening are the best means of preventing unwanted encounters caused by disturbing colonies.

Common species include *Bombus impatiens* Cresson (Fig. 2.15) and *B. bimaculatus* Cresson in the eastern half of the United States and southeastern Canada, with *B. ternarius* Say (Fig. 2.16) in the northern part of this region, *B. bifarius* Cresson, *huntii* Greene, and *morrisoni* Cresson in the Rocky Mountain region and southwest, and *B. vosnesenskii* Radoszkowski (Fig. 2.17) and *B. melanopygus* Nylander (the dark *edwardsii* form) along the Pacific Coast. Another species widespread in the east but extending across the United States toward the Pacific Northwest is *Bombus griseocollis* (DeGeer). The number of species just mentioned should be much greater, but due to one or more factors (particularly the possible introduction of pathogens from other continents), a number of previously abundant and widespread species of *Bombus* have become rare with a couple being considered in danger of extinction. This has implications for the pollination and, hence, survival of various species of plants [29]. Queen bumble bees are less likely to attack than workers, but they are larger and possess longer stings and bigger venom sacs and thus are capable of inflicting more pain.

Several families of solitary bees have females capable of stinging if confined or squeezed against the skin. Two of these are of note—one due to its tendency to sting



Fig. 2.15 *Bombus impatiens* queen. https://upload.wikimedia.org/wikipedia/commons/e/e9/B_impatiens_queen.jpg (Photographer: Ilorban 3008 × 2000 pixels. License: Creative Commons Attribution-Share Alike 3.0 Unported)



Fig. 2.16 *Bombus ternarius* worker. https://commons.wikimedia.org/wiki/File:Bombus_ternarius.jpg (Photographer: Beatriz Moisset 550 × 452 pixels. License: Creative Commons Attribution-Share Alike 2.5 Generic)



Fig. 2.17 *Bombus vosnesenskii* worker. https://upload.wikimedia.org/wikipedia/commons/2/21/Bombus_vosnesenkii.jpg (Photographer: Kevin Cole 4200 × 2874 pixels. License: Creative Commons Attribution-Share Alike 2.0 Generic)



Fig. 2.18 *Xylocopa virginica* (carpenter bee) female. https://upload.wikimedia.org/wikipedia/commons/7/7e/Xylocopa_virginica-Eastern_carpenter_bee_(DSC_0505cr).JPG (Photographer: ysmad.com 4430 × 3730 pixels. License: Creative Commons Attribution-Share Alike 3.0 Unported)

and the other because of its conspicuousness. The sweat bees (genera *Halictus* and *Lasioglossum* [30] and perhaps other genera in the family Halictidae) are small, typically about 5 mm in length, and some species have developed a form of sociality. Although the insects are unaggressive and have mild stings, their tendency to land on human skin to lap perspiration causes them to be squeezed inadvertently, resulting in defensive stings.

The other notable genus of bees is *Xylocopa* which is comprised of the large carpenter bees (now in family Apidae, subfamily Xylocopinae [31]; these bees used to be placed within the family Anthophoridae with the digger bees). These large bees superficially resemble queen bumble bees but have large heads and shiny abdomens due to having less hair (Fig. 2.18). Although they are somewhat gregarious, their life cycle is solitary with no worker or queen castes among the females. They are capable of stinging, but despite their large size and presence around buildings, causing them sometimes to be perceived as threatening, they are unaggressive.

Preventing Stings

A number of websites (e.g., one provided by the Mayo Clinic [32]) offer suggestions regarding the prevention of stings, with some being more helpful than others. Some additional practical comments regarding the prevention of stings, gained from field experience, are in order. Many cases of stinging, particularly multiple stings, result from disturbances to colonies. Although any given species may build a nest in a wide range of settings, each species has certain "favorite" locations. For example, several species of *Vespula*, particularly those in the *V. vulgaris* group, are commonly found along road and stream banks where the sloping soil often provides exposed roots, crevices between layers of rocks, soil slumps, and animal burrows, any of which can be inviting opportunities for searching yellow jacket queens, and the road or stream usually provides the insects an easy opportunity for visual discovery of such potential nesting sites. Abandoned buildings provide shelter for *Polistes*, *Vespula*, and even some *Dolichovespula* if there are broken windows, open doors, or spaces between boards, all of which can serve as entrance points. The exterior provides additional nesting sites, especially under the eaves (favored by *Polistes* and *Dolichovespula*) or openings in the outside wall that lead to cavities within the wall (favored by *Vespula*, *Vespa crabro*, and honey bees). Farms provide many nesting spaces for nearly the entire range of species present in a given area; for example, spaces between bales of hay may provide nesting site for bumble bees, intact and occupied houses may have eaves or overhangs that invite *Polistes* and *Dolichovespula*, and the landscaping may provide nesting sites for *Vespula*. In addition, the branches of essentially any tree or shrub can support a colony of *Dolichovespula*, and hollow trees can harbor honey bees or European hornets. If a colony of feral honey bees is discovered, the possibility of its being of the Africanized strain should be considered if one is in the southern and southwestern areas of the United States, so it is best to avoid approaching it. Therefore, one should always take into account the possibility of a nest being nearly anywhere, with special attention to the situations described above. Any small space may serve as a nesting site for a colony of *Polistes*, and because of the small colonies produced by this genus (compared to vespines), there probably will not be enough activity to announce the presence of a colony. The author once got stung when opening the lid to the gas filler cap in a car that hadn't been driven for a few summer months—a nest of *Polistes fuscatus* had been built near the cap during that time!

Although one is unlikely to squeeze a stinging insect purposely (except for small children who are naive as to the consequences!), situations occur in which one may inadvertently do so. For example, food or drink eaten outdoors may be attractive to yellow jackets, and even honey bees will seek sweet drinks, particularly on warm days late in the season when sources of nectar become scarce. One can get stung on the hand by squeezing the insect when picking up the cup or food item or stung in or on the mouth when eating or drinking is attempted. Lawns and fields, especially those supporting flowers, can present a hazard to anyone walking through them with floppy clothes capable of trapping insects underneath. If a bee becomes trapped

inside a trouser leg, pinching off a section of the fabric to isolate the bee away from the skin and then squeezing this section at each end a short distance from the bee will kill it such that it can drop harmlessly to the ground—a procedure successfully undertaken by the author against an unfortunate bumble bee.

As was mentioned above, avoidance of stings from imported fire ants is quite easy in most, if not all, situations, mainly by being careful not to disturb their mounds. Large colonies are easy to recognize, but small ones are less so; thus, when in fire ant country, any loose sand that appears different from the "background" soil should be suspected of hosting fire ants. However, small children, certain disabled people, and pets are at risk of disturbing fire ant mounds, so supervision by a responsible adult is necessary. In addition, the occasional colony may become established in structures or other locations where a nest may not have an obvious mound. Colonies of harvester ants are usually easy to recognize, so they should be avoided easily. Although some species of harvester ants are unaggressive, they may climb up a person's leg if one is standing near their nest, and pressure from clothes or inadvertent squeezing is likely to result in being stung.

Quick movements for the most part are best avoided when social bees or wasps are flying about a person, but two exceptions exist. The first is that if a single insect is threatening and one is confident in his ability to kill or at least immobilize it, then striking the culprit with a well-aimed firm object (or netting it) may prevent a sting. This action assumes one is far enough from the nest that nest mates will not become involved either due to visual or chemical cues (the latter due to pheromones, discussed below). The second is that if one is about to be attacked by numerous stinging insects, removing oneself from the immediate vicinity of the angry colony by running away will prevent most if not all stings, especially if one runs a few hundred feet preferably with some intervening vegetation causing the insects to lose sight of the runner [33].

Some members of the genus *Vespula* are scavengers, and workers of these species often approach people in search of food. A yellow jacket's back-and-forth movements about one's hands, when one is not near a nest, are simply part of its investigating a potential food source, particularly if one has been handing any item that attracts its interest. For example, workers of *V. maculifrons* have been observed landing on a hand after a meal including shrimp had been eaten, and the wasps even bit (i.e., engaged their mandibles) in an apparent attempt to remove the skin from the hand but soon lost interest and left. If wasps are persistent, it is best to move away slowly and enter a vehicle or building if possible.

Stinging insects, in particular social species, release alarm pheromones—chemicals whose purpose is to alert their nest mates to the presence of an intruder perceived as a threat to their colony. The particular compounds released vary considerably among the species, and some are easily detected by the human nose. For example, the alarm pheromone of the European honey bee is usually described as having an odor like banana oil, while that of the yellow jacket *Vespula squamosa* of the southeastern United States reminds one of brown sugar or dried coffee. Most other alarm pheromones have more subtle odors (at least to the human nose), but their release upon stinging increases the likelihood of additional stinging, particularly

in the vicinity of the nest, communicating "I've just marked the enemy—let's all charge!" Therefore, one should attempt to put distance between the nest and himself after having disturbed it, especially if a sting has already been received.

Finally, one who is hypersensitive to stings should not attempt to exterminate or remove nests but leave this task to others, including professional exterminators if necessary. In the author's experience, a nest that has been damaged due to an unsuccessful attempt at extermination is likely to be more defensive than one that has been left undisturbed.

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Chapter 3
Hymenoptera Venoms: Toxicity, Components, Stability, and Standardization

Ellen L. Danneels, Matthias Van Vaerenbergh, and Dirk C. de Graaf

The Venom Apparatus: An Evolutionary Perspective

Venoms are toxic substances containing a broad range of compounds including organic molecules, amines and alkaloids, salts and minerals, amino acids, peptides, and proteins [1]. A specialized apparatus, such as a stinger, fang, hollow spine, or other mechanical delivery system, injects these compounds into the victim [1, 2]. Venomous animals include sea anemones, jellyfish, gastropods, cephalopods, centipedes, insects, echinoderms, amphibians, reptiles, fish, and five mammalian species. The venom composition, its delivery system, and physiological target can differ considerably, as these venoms serve different functions and evolved independently between the phylogenetically divergent lineages [1].

When people think of insects, images of stings and bites often flash into mind. Venoms are widely distributed throughout the class of insects, but the ants, wasps, and bees belonging to the order of Hymenoptera are the characteristic groups of venomous insects [2]. Remarkably, hymenopterans share a common parasitic ancestral origin [3]. Many parasitoid wasps of the Terebrantia (paraphyletic suborder [4]) still use their stinging organ (terebra) to deposit their eggs inside (endoparasitoids) or outside (ectoparasitoids) the body cavity of invertebrate hosts. In addition, venom is released which can target the host's immunity, physiology, mobility, reproductive capacity, and even their behavior in order to guarantee the offspring's development [3, 5]. The venom composition in parasitoids varies between species, even within the same genus, reflecting their important functional diversification during evolution [3]. In contrast, in the Aculeata, a monophyletic suborder derived within the Terebrantia [4], the ancestral ovipositor has evolved to a highly specialized device

E.L. Danneels, Ph.D. (✉) • M. Van Vaerenbergh, Ph.D. • D.C. de Graaf, Ph.D.
Laboratory of Molecular Entomology and Bee Pathology, Department of Biochemistry and Microbiology, Ghent University, Krijgslaan 281 S2, 9000 Ghent, Belgium
e-mail: Ellen.Danneels@UGent.be; matthiasvanvaerenbergh@hotmail.com; Dirk.deGraaf@UGent.be

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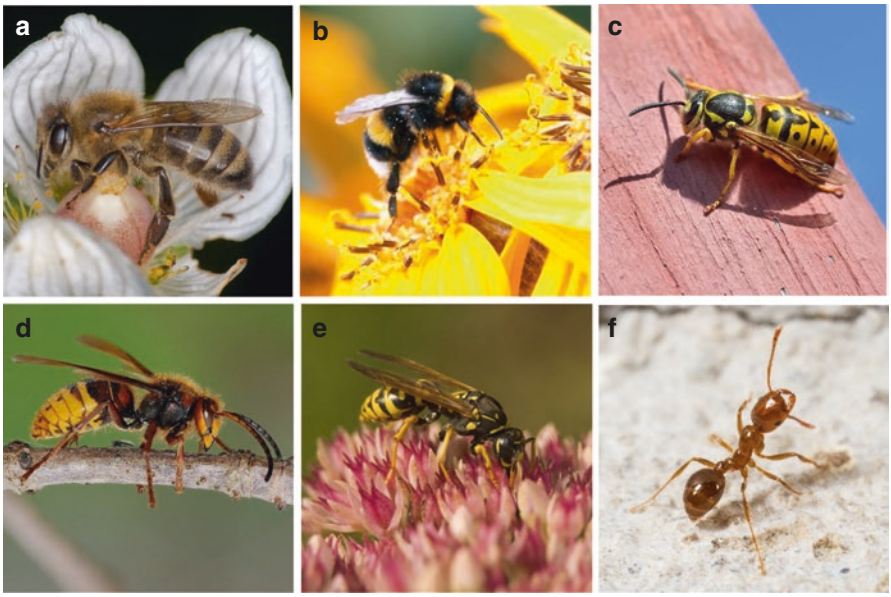


Fig. 3.1 Some stinging hymenopteran species: *Apis mellifera* (**a**), *Bombus terrestris* (**b**), *Vespula vulgaris* (**c**), *Vespa crabro* (**d**), *Polistes dominulus* (**e**), *Solenopsis invicta* (**f**). Figure adapted from [7]. Pictures remain copyright of Honeybee Valley, Ghent University

(aculeus) for injection of venom and no longer serves for the egg-laying function, except in more basal lineages (e.g. Dryinidae and Chrysididae) [3]. Only species belonging to three families of Hymenoptera sting humans with a high frequency: Vespidae (wasps), Apidae (bees), and Formicidae (ants) [6, 7]. Vespids include the genera *Vespula* (yellow jackets), *Dolichovespula* (aerial yellow jackets), *Vespa* (hornets), and *Polistes* (paper wasps), while aphids include the genera *Apis* (honeybees) and *Bombus* (bumblebees). Also few ant species belonging to the genera *Solenopsis* (fire ants) and *Pogonomyrmex* (harvester ants) retained their stinger and are capable of delivering painful stings. Some well-known stinging hymenopteran species are depicted in Fig. 3.1. While some of the aculeate species use venom only for prey capture purposes, others are also effective against predators, including humans [2]. In contrast, bees no longer have a predatory lifestyle and shifted to a diet of pollen and nectar. Therefore, the major function of their venom evolved to personal defense or defense of the colony by inflicting pain. As the possession of a venomous sting is unique in its power against very large enemies, it is believed to have facilitated the evolution toward eusociality, the condition of living in colonies with cooperative brood care, reproductive castes, and overlapping of adult generation, which has evolved multiple times within the Aculeata [8]. Remarkably, some eusocial living bees have lost a functional stinger through evolution. For example, the Meliponini are stingless bees which have acquired defensive behavior which differs from that of the *Apis* species. While both queens and workers of primitive stingless bee species retained the venom sac homologues of stinging bees, only the queens of evolved meliponine species have preserved this vestigial character [9].

Relation Between Venom Apparatus and Life Cycle

In the Aculeata, the ovipositor is fully developed as a venom apparatus and is usually no longer associated with oviposition. At the posterior section of the abdomen, the venom apparatus is located which consists of glands and a complex chitinous sting apparatus. The basic sting apparatus structure is anatomically similar for all Aculeata, consisting of a long, thin, distally bifurcated venom gland which produces the venom compounds (Fig. 3.2). Venom glands are epidermal glands that have evolved from female accessory reproductive glands [10]. Venom compounds are stored in the venom sac/reservoir and are released upon stinging. However, the life cycles of bees, wasps, and ants differ. As this can have significant implications for the venom composition and sting apparatus, relevant differences in life cycle for stinging insects that regularly sting humans are described.

Honeybees that belong to the Apidae live in large hives, which can reach up to 80,000 individuals during the summer months. Only one queen is present in the hive which lays the eggs, while workers are sterile and engage in various tasks depending on their age. While drones lack a stinger, both the queen and workers possess a morphologically distinct stinger, which is used for a different purpose. Workers use their venom for defending the hive, which is a rewarding target for many predators searching for honey, pollen, immature brood, and adults. When a bee colony is threatened with attack, bees at the nest entrance or at the periphery of a cluster elevate their abdomens and protrude their stings, thereby releasing alarm pheromones. This alerts the entire colony. Alarm pheromones are also released by a sting left embedded in the body of an intruding mammal, thus guiding other bees to the victim [13]. The stinger of workers is barbed and possesses an associated set of muscles and nerves, allowing to penetrate the target deeper independent of the rest of the body (Fig. 3.3). In case of stinging mammals with a thick skin, the stinger is torn from the body, and the muscles surrounding the venom sac will continue to pump

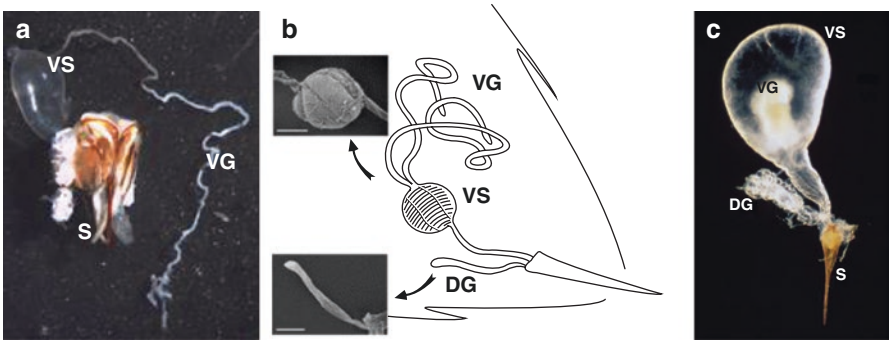


Fig. 3.2 Morphology of the sting apparatus representative for the Apidae, Vespidae, and Formicidae: *Apis mellifera* (**a**) and *Dolichovespula media* (**b**) figure adapted from [11], *Solenopsis* sp. (**c**) figure adapted from [12]. Venom is produced by the venom glands, stored in the venom sac, and released upon stinging via the stinger and its associated set of muscles and nerves. *VG* venom gland, VS venom sac, *DG* Dufour gland, *S* stinger

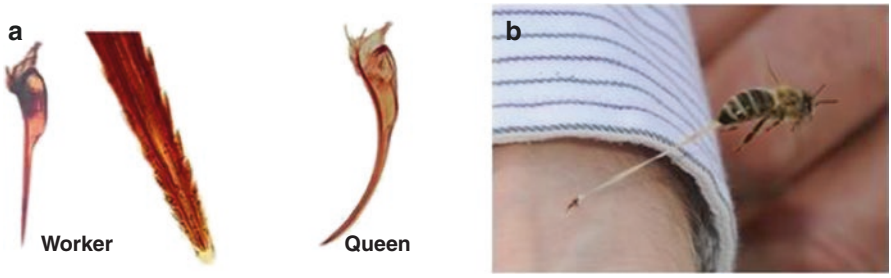


Fig. 3.3 (**a**) Morphology of the stinger of the honeybee worker and queen. The stinger of the workers is straight and barbed, while the queen stinger is curved and smooth. (**b**) Upon stinging, the barbs present on the stinger of workers cause the release of the sting apparatus, which maximizes venom delivery. Picture **b** remains copyright of Kathy Keatley Garvey

venom into the attacker. This process of autotomy maximizes venom delivery [2]. The venom sac of European honeybees contains about 150 μg of dry weight venom protein, which is almost completely delivered from the sting within 1 min [14]. However, tearing the stinger loose damages the honeybee's abdomen which leads to its death. In contrast, queens use their stinger only in deadly fights with rival queens [15]. Therefore, their stinger is curved and smooth which allows them to sting multiple times (Fig. 3.3). Both the venom gland and reservoir are much larger than those of workers, enabling the storage of up to five times more venom [16]. Queen venom has been reported to be only half as lethal to mice as worker venom [17], which points to a different venom composition. Recently, the queen venom proteome was investigated which showed the presence of two queen-specific venom proteins and the lack of several worker-specific venom proteins [18]. Moreover, the start of venom gland activity differs between both castes which has evolved due to the fact that queens and workers need a functional venom at a different time in their life [19]. Queens use the venom upon emergence to fight with other queens. They can live up to 5 years, but by the time they reach the age of 1–2 years, their venom has become inactive [17]. In contrast, workers use the venom when performing tasks outside the hive, which starts around the 20th day of adult life. The venom glands of both castes have only one secretory cycle, which starts at the end of pupation in queens and just after emergence in workers. In workers, the highest secretory activity of the venom glands is reached around the 16th day. Therefore, the venom composition is also age dependent. In addition to these quantitative and qualitative caste- and age-related alterations, seasonal variation in venom composition has been demonstrated [18, 20]. Also variability due to geographical and colony-dependent factors may exist in honeybee venom, but this has not been studied in depth.

Bumblebee nests are generally much less extensive than those of honeybees. As the queen is the only individual surviving through the winter, these are the first ones you see during spring. The queen constructs a nest (usually underground) and lays eggs developing in workers which forage and live for several weeks. By the end of the summer, new queens and drones develop which mate. Only the young queens

will survive the winter through diapause. Although the amount of venom released during a sting varies from species to species and within a species, generally, the amount of venom protein released by a honeybee sting is approximately five times greater than that released by a bumblebee sting [21]. However, in contrast to honeybees, both the bumblebee queens and workers possess a smooth and curved stinger which is strongly attached and enables them to sting more than once [22]. In contrast to honeybee venom which has received much interest the last few decades, bumblebee venom has only recently been investigated using mass spectrometric approaches [23].

Wasps (Vespidae) are social insects, forming colonies and building their nests underground, in trees, hedges, and walls and roof spaces. The queen emerges during spring and starts to build a nest, using papery material she makes by chewing small pieces of wood mixed with saliva. She lays the eggs that later on emerge into sterile worker wasps. Nest building continues until the colony contains many hundreds to thousands of workers. Wasps use their sting to paralyze their prey and as a defense mechanism. Unlike bees, a wasp can extract its smooth stinger from the victim's skin and fly off. Due to their capability of repeated stings, Vespidae generally inject less venom, ranging from 1.7 to 17 μg of protein per sting [21]. The venom composition of vespids has been intensively studied, and multiple allergens have been determined (see Table 3.1). Due to similarities of venom composition and of the structure of individual allergens, cross-reactivity among vespids is strong [24]. Many studies on social wasps have also shown that a chemically volatile fraction of the venom, generally containing spiroacetals, amides, and acetates of saturated mono- and di-unsaturated 2-alcohols, takes part in colony defense, acting as an alarm pheromone. The presence of pheromones in the venom, eliciting alarm responses and attracting nest mates on the nest surface or toward potential enemies, has been demonstrated for species of the subfamily Vespinae and Polistinae [25]. Pheromones with similar functions are also present in venoms of the following group of insects.

Ants (Formicidae) also form colonies, but they can largely differ in size, ranging from a few dozen predatory individuals living in small natural cavities to highly organized colonies that may occupy large territories and consist of millions of individuals. Larger colonies consist mostly of sterile, wingless females forming castes of workers, soldiers, or other specialized groups. Nearly all ant colonies also have some fertile males called drones and one or more fertile females called queens. All ants, except the ones belonging to the subfamilies Formicinae, Dolichoderinae, Aneuretinae, and Dorylinae, are able to sting, using their venom for several purposes such as defense against predators/competitors and microbial pathogens, for predation, as well as for social communication [26]. The evolution of venom has enabled ants to develop a venom composition that is algesic and lethal, enabling them to defend their nest and the queen against large intelligent vertebrate predators. Some ant stings are known to be extremely painful for humans, for example, stings by fire or bullet ants. In particular, the latter have been classified as producing the most painful sting among all Hymenoptera and the third most painful sting of all venomous animals [8].

Table 3.1 All Hymenoptera venom allergens from the official list of allergens of the IUIS (http://www.allergen.org) are shown

Family	Genus	Protein name	Allergen name						
Apidae	*Apis*		*A. mellifera*	*A. cerana*	*A. dorsata*				
		Phospholipase A_2	Api m 1	Api c 1	Api d 1				
		Hyaluronidase	Api m 2						
		Acid phosphatase	Api m 3						
		Melittin	Api m 4						
		Dipeptidyl peptidase IV	Api m 5						
		Protease inhibitor	Api m 6						
		CUB serine protease	Api m 7						
		Carboxylesterase	Api m 8						
		Serine carboxypeptidase	Api m 9						
		Icarapin	Api m 10						
		MRJP8 and MRJP9	Api m 11						
		Vitellogenin	Api m 12						
	Bombus		*B. terrestris*	*B. pensylvanicus*					
		Phospholipase A_2	Bom t 1	Bom p 1					
		Protease	Bom t 4	Bom p 4					

Vespidae	*Vespula*		*V. vulgaris*	*V. maculifrons*	*V. squamosa*	*V. flavopilosa*	*V. germanica*	*V. pensylvanica*	*V. vidua*
		Phospholipase A_1	Ves v 1	Ves m 1	Ves s 1				
		Hyaluronidase	Ves v 2	Ves m 2					
		Dipeptidyl peptidase IV	Ves v 3						
		Antigen 5	Ves v 5	Ves m 5	Ves s 5	Ves f 5	Ves g 5	Ves p 5	Ves vi 5
		Vitellogenin	Ves v 6						
	Dolichovespula		*D. maculata*	*D. arenaria*					
		Phospholipase A_1	Dol m 1						
		Hyaluronidase	Dol m 2						
		Antigen 5	Dol m 5	Dol a 5					
	Vespa		*V. crabro*	*V. mandarinia*	*V. magnifica*				
		Phospholipase A_1	Vesp c 1	Vesp m 1					
		Hyaluronidase			Vesp ma 2				
		Antigen 5	Vesp c 5	Vesp m 5	Vesp ma 5				
	Polistes		*P. dominulus*	*P. exclamans*	*P. annularis*	*P. gallicus*	*P. fuscatus*	*P. metricus*	
		Phospholipase A_1	Pol d 1	Pol e 1	Pol a 1	Pol g 1			
		Hyaluronidase			Pol a 2				
		Serine protease	Pol d 4	Pol e 4					
		Antigen 5	Pol d 5	Pol e 5	Pol a 5	Pol g 5	Pol f 5	Pol m 5	
	Polybia		*P. paulista*	*P. scutellaris*					
		Phospholipase A_1	Poly p 1						
		Antigen 5		Poly s 5					

(continued)

Table 3.1 (continued)

Family	Genus	Protein name	Allergen name						
Formicidae	*Myrmecia*		*M. pilosula*						
		Pilosulin-1	Myr p 1						
		Pilosulin-3	Myr p 2						
		Pilosulin-4	Myr p 3						
	Solenopsis		*S. invicta*	*S. geminata*	*S. richteri*	*S. saevissima*			
		Phospholipase A_1	Sol i 1						
			Sol i 2	Sol g 2	Sol r 2	Sol s 2			
		Cystein-rich venom protein	Sol i 3	Sol g 3	Sol r 3	Sol s 3			
			Sol i 4	Sol g 4					

Homologous allergens within each genus are presented per protein name

Toxicity of Hymenoptera Venom

A single honeybee sting induces death in other insects and pain and inflammation in higher organisms. However, when (accidentally) disturbing a honeybee colony, it responds aggressively which can result in mass stinging events. Between different colonies, striking variation in the intensity of the aggressive response is noticeable. In docile colonies only a few bees may respond, whereas in more aggressive colonies, the response may involve hundreds or even thousands of stinging individuals [27]. The most severe aggressive behavior is observed in the Africanized honeybee (AHB), originated in Brazil by introduction and crossbreeding of African honeybees (*Apis mellifera scutellata*) with European honeybees (predominantly *A. mellifera ligustica*) [27]. AHBs reached the wild in Brazil in 1957 and spread north and south. They appear to produce lower amounts of venom, and the general biochemical composition and lethality of their venom does not differ from their European counterparts [28]. However, AHBs have been found to sting human airways more often and are able to release higher amounts of venom upon stinging, suggesting a reason for fatalities from relatively small numbers of stings [29]. Since bee stings have become a public health concern in the American continent, where massive attacks by AHBs in humans and animals have been documented, interest in the physiopathological effects of honeybee venom increased [28]. Mass envenomations lead to systemic toxicity with a potential fatal outcome. Receiving more than 500 stings is usually fatal, although many victims have survived more than 1000 honeybee stings by receiving medical treatment [29, 30]. A series of acute toxic effects were described in mice by inducing a severe, sublethal systemic envenomation in response to honeybee venom subcutaneous injections. A variety of increased biochemical markers revealed liver, skeletal muscle, and kidney damage. Also disturbances in the coagulation system and a hemoconcentration (hematocrit and hemoglobin increase) effect were observed, while circulating platelet and leukocyte numbers remained unaltered. In addition, an inflammatory response including edema, lipid peroxidation, nitric oxide production, and systemic release of cytokines (IL-1β, IL-6, TNF-α) was demonstrated. These findings seem to be in concordance with the reported clinical effects in humans [28]. Immediate effects include localized pain, swelling, and erythema at individual sites. Next, large envenomations cause early systemic symptoms which include fatigue, dizziness, nausea, vomiting, and diarrhea. Within 24 h, hemolysis, hemoglobinuria, rhabdomyolysis, and hepatic transaminase enzyme elevations develop. Also subendocardial damage and cardiac enzyme elevation may occur, while renal insufficiency and electrolyte abnormalities develop secondary to rhabdomyolysis, hemolysis, and acute tubular necrosis [31]. The search for an effective treatment for bee envenomation has been the subject of many studies. Already in 1999, Jones et al. [32] produced the first antivenom against AHB venom by immunizing sheep. However, the use of heterologous sera can cause anaphylaxis and serum sickness. Recently, a new antivenom was developed by the use of phage display technology, which enabled the production of human antibody fragments binding to two major honeybee venom

compounds [33]. As currently no commercially available antivenom exists, massive envenomations have to be treated with supportive care, involving antihistamines, steroids, epinephrine, and airway assistance. Massive bee envenomations by other honeybee subspecies are less common but have been described in regions such as England, India, and Hawaii, where Africanized bees are not endemic [31]. Venoms of several species of the *Apis* genus (*A. mellifera*, *A. dorsata*, *A. cerana*, *A. florea*) exhibit an almost identical median lethal dose (LD50) for mice [17], which indicates that their venom composition is very similar. Subcutaneously injected honeybee venom has an LD50 of 41.6 mg/kg, which is nearly one order of magnitude weaker in comparison to LD50 values obtained by intravenous or intraperitoneal routes. This suggests that a substantial fraction of the lethal components of bee venom are prevented from entering the systemic circulation when administered subcutaneously, which may be caused by venom inactivation by host factors and/or binding to locally available tissue target sites [22].

Compared to honeybees, bumblebees rarely sting humans. Intraperitoneal injection of venom of the bumblebee *Bombus impatiens* (LD50 = 7.2 mg/kg) in mice showed that it is about half as lethal to vertebrates as honeybee venom (3.5 mg/kg) [29]. However, the venom composition of this bumblebee species has not yet been investigated. The LD50 of the venom of *B. terrestris* has not yet been determined.

Aculeate venom composition underwent evolution toward producing toxins that would be more effective against potential attackers. Usually, solitary wasp venoms are employed primarily to paralyze and then kill prey [34]. Additionally, wasp venoms are used to defend the colony against foreign individuals. On mammals, vespid venoms provoke prolonged pain, local edema, and erythema due to increased permeability of blood vessels in the skin. Besides these direct outcomes of hornet stings, allergic reactions have also been observed in numerous cases. The generalized allergic reaction may be lethal. In addition to their systemic effects, wasp and hornet venoms act kinetically on isolated smooth muscle and reduce blood pressure. They release endogenous histamines from granulocytes including mast cells and basophilic leukocytes and also release catecholamines from adrenal chromaffin cells. Such toxins may also provoke cytolysis, including hemolysis and chemotaxis to macrophages and polymorphonuclear leukocytes. Massive stings by wasp swarms, however, can cause toxic reactions and multiple organ dysfunction rather than an anaphylactic reaction [35]. Acute kidney injury is the prominent clinical manifestation with high creatinine levels, shock, oliguria, and anemia that are risk factors for death. Fatal stings usually occur in the head or neck, and less commonly, death occurs from the toxic effects of massive envenomation involving hundreds of stings [36]. On experimental rodents, LD50 of the venom of the European hornet *V. crabro* is 8.7–10.9 mg/kg. However, when *V. crabro* venom is applied on *Calliphora* sp. larvae, the toxicity is increased to 2.7–7.6 mg/kg, showing a higher toxicity in insects compared to vertebrates. Wasps have also been shown to use their venom apparatus effectively when attacking foreign workers that appear in the immediate vicinity of their nest (LD50 = 4.0 mg/kg) [37].

Stings from stinging ants, like *Solenopsis* (fire ants), *Pachycondyla* (ponerine ants), *Myrmecia* (bulldog ants), and *Paraponera* (bullet ants), are usually multiple,

because ants tend to attack en masse and sting repeatedly. When disturbed, the imported fire ant produces painful lesions with its unique method of stinging. The fire ant attaches itself to the victim with its jaws and then arches its body to insert a lancet-like stinger from the distal abdomen into the skin [38]. The ant holds this position for about 20–25 s and then may rotate its head and reinsert in two or three additional sites [39]. Each sting may deliver 0.04–0.11 μL of venom, which induces immediate pain at the site of injection [38]. Local itching may be intense and could last for hours. Death caused by ant stings is rare, although some stung experience life-threatening anaphylaxis [38]. Some ants have developed venoms primarily for defense against vertebrates, like the venom from *Pogonomyrmex badius* that is highly toxic toward mice, but not very toxic toward insects. It is clear that ants have evolved venoms containing numerous toxins to induce pain, discomfort, paralysis, and/or death in vertebrate and arthropod predators or prey [40].

Biological Function of Hymenoptera Venom Compounds

Knowledge of the composition of venoms and the structure of allergens is a prerequisite for the accurate diagnosis and treatment of insect venom allergy. Several Hymenoptera venom allergens have been reported and are named following the nomenclature guidelines of the International Union of Immunological Societies (IUIS; http://www.allergen.org) (Table 3.1).

Hymenoptera Venom Enzymes

One relatively common component in Hymenoptera venoms is phospholipase, mainly occurring as –A and –B types [41]. Phospholipases A_1 (PLA_1) and A_2 (PLA_2) catalyze the specific hydrolysis of ester bonds of 1,2-diacyl-3-sn glycerophospholipids sn-1 and sn-2, respectively, converting these substrates into their corresponding lyso compounds with the release of fatty acids [42]. Thus, the PLAs are able to disrupt the phospholipid wrappings of biological membranes, leading to pore formation, cell lysis, inflammation, and tissue damage. When compared to each other, PLA_1s and PLA_2s have no sequence homology; apparently, these proteins have distinct functions. PLA_2 was previously reported in social Hymenoptera venoms and described as highly hemolytic [43]. Generally, PLA_2 from wasp and bee venoms are reported as allergenic proteins. Venom from *A. mellifera* and *B. terrestris* both contain two group III PLA_2s of which phospholipase A_2-1 is a known allergen in both species, Api m 1 and Bom t 1, respectively [44, 45]. Recently, Fourier transform mass spectrometry (FTMS) revealed a third PLA_2 compound in the venom of *A. mellifera*, group XV PLA_2 [46]. PLA_1 has been partially characterized in the venoms of some wasp species from the Northern Hemisphere [47], in ants [48], and in the neotropical social wasp *Polybia paulista* [49]. The PLA_1s are generally

associated with allergic and inflammatory processes [47]. As for several other venom compounds, the enzymes PLA_1 and PLA_2 occur as series of multiple proteins, suggesting that they occur as a series of different isoforms and/or posttranslationally modified forms [50]. Phospholipase B (PLB) has also been found in hymenopteran venoms, more particularly in hornets [51] and harvester ants [52]. PLB, which cleaves both sn-1 and sn-2 acyl chains and is involved in the degradation of phospholipids, can cause the lysis of erythrocytes and mast cells and contributes to the venom toxicity. In hornets, the venom is not only used as toxins for preyed insects, but it also digests their cell wall components of diacylphospholipid, such as phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine, to fatty acids and phospholipids by containing phospholipases. PLBs are stronger digestive enzymes than PLA_1 or PLA_2, because A1 and A2 sites are hydrolyzed by one enzyme. Carnivorous insects, like *Vespa*, seem to have this enzymatic adaptation in their venom, while vegetarian insects, like *Apis*, only contain PLA_2 in the venom. Many PLBs from hornets and wasps are known to be unstable for higher pH, freeze-thawing, and room temperature [53]. Proteomic study on the venom from the fire ant *S. invicta* revealed the presence of a phospholipase D1, which until then was not known as a component in any animal venom [48].

The second well-known hymenopteran venom allergen is hyaluronidase. This glycolysis hydrolase cleaves hyaluronic acid, which is a large and highly abundant glycosaminoglycan of the vertebrate extracellular matrix. This cleavage facilitates the penetration of venom constituents into the body [54]. Hyaluronidase has been recognized as major allergen (Api m 2) in *A. mellifera* venom [6]. In the aggressive social wasp *P. paulista*, hyaluronidase was suggested to be the primary venom protein that is responsible for triggering allergic symptoms caused after accidents with this wasp [55]. However, hyaluronidase has recently been shown to be only a minor vespid allergen, at least in Vespula-allergic subjects [56]. Most of the IgE reactivity seen with hyaluronidase is directed against cross-reactive carbohydrate determinants (CCDs), and binding to hyaluronidase is seen almost exclusively in wasp–honeybee double-positive patients [57, 58]. Hyaluronidases represent one of the most strongly conserved Hymenoptera allergens (see Table 3.1) and are also thought to be as one of the most important cross-reactive allergens in yellow jacket and honeybee venom [6]. More specifically, Vesp ma 2, the hyaluronidase allergen in the hornet *V. magnifica*, shares 92%, 91%, 73%, 54%, and 41% sequence similarity with the Dol m 2 from *D. maculata* [59], Ves v 2 from *V. vulgaris* [60], Pol a 2 from *P. annularis* [61], and Api m 2 from *A. mellifera* [62], respectively.

When studying the enzymes in Hymenoptera venoms, the presence of acid phosphatase exhibits a distinct correlation with taxonomy. None of the social or solitary wasp venoms possess acid phosphatase, whereas all the ants and the bees do show acid phosphatase activity in their venoms [63]. Only in the honeybee, acid phosphatase or Api m 3 has been recognized as an allergen by the IUIS (see Table 3.1). Interestingly, ant venoms contain significantly higher levels of acid phosphatase than social wasp venoms. Whether or not this is linked to the fact that ant venoms are generally, but not significantly, more lethal still needs to be elucidated.

Several serine proteases are found in hymenopteran venoms. They belong to the trypsin family S1 of clan SA, the largest family of peptidases [64]. Many serine proteases have been found from animal venoms, especially from snakes. They can affect the hemostatic system by acting on a variety of components of the coagulation cascade, on the fibrinolytic and kallikrein–kinin systems, and on cells [65]. Serine proteases have also been found in bumblebee [23], honeybee [46], hornet [66], and paper wasp venoms [67]. Honeybee venom contains five serine proteases with different binding domains (no domain/CUB/CLIP), which may allow a similar catalytic activity, but each directed toward a specific target [18, 46]. This broadens the panel of toxins that allows the honeybees to efficiently defend the hive. Recently, a sixth serine proteinase was found in venom of honeybee queens, more specifically serine proteinase stubble, which could not be detected by FTMS in worker venom [18]. One of the serine proteases in honeybee venom is known as allergen Api m 7 (see Table 3.1) and represents a combination of structurally and functionally different modules: a CUB domain is connected through a linker peptide with a serine protease-like (SPL) domain [68]. The enzymatic properties of the allergen are mediated by the SPL domain, while the N-terminal peptide serves as an allergenic determinant. The CUB domain mediates protein–protein interactions during development and in interacting protein cascades like the classical complement pathway [69]. Proteomic analysis of bumblebee venom revealed the presence of multiple serine proteases. Five of these, serine proteases 1–5, show high mutual similarity, and all are similar to the honeybee venom CLIP serine protease [23]. A sixth serine protease (Bi-VSP) was described to be present in bumblebee (*B. ignitus*) venom that has an arthropod prophenoloxidase(proPO)-activating factor (PPAF) function and fibrinolytic activity [70]. In some insects, Bi-VSP triggers the phenoloxidase (PO) cascade by the activation of PPAF. Injection of purified Bi-VSP induces a lethal melanization response in target insects by modulating the innate immune response. In mammals, Bi-VSP acts similarly to snake venom serine protease [71], which exhibits mammalian fibrinolytic activity. The fibrinolytic activity of Bi-VSP, possibly inhibiting blood coagulation, can facilitate the spread of toxic components throughout the bloodstream. Additionally, when integrating the multifunctional Bi-VSP to the insect-killing fungus *Beauveria bassiana*, fast melanization of whole insect bodies was induced, and the transformants had much higher insecticidal potency [72]. Serine protease allergens Pol d 4 and Pol e 4 have also been identified in paper wasps *Polistes dominulus* and *P. exclamans*, respectively (see Table 3.1) [67]. Venom of the hornet *V. magnifica* contains magnvesin, a toxin that shows serine protease-like activity and inhibits blood coagulation by hydrolyzing coagulant factors TF, VII, VIII, IX, and X [66]. It shares 52% identity with allergen serine protease from the wasp *P. dominulus*.

A venom compound that is found in Apidae as well as in Vespidae is the highly glycosylated serine protease dipeptidyl peptidase IV (DPP IV). In *A. mellifera* and *V. vulgaris*, both are known as allergens Api m 5 and Ves v 3, respectively (see Table 3.1). Insect venom DPP IV may function through the conversion of venom components into their active forms in the venom gland on the one hand and the enhancement or decrease of the chemotactic activity of immune cells after the insect

sting on the other hand. The former may be confirmed by the hypothesis that promelittin in insect venoms is processed into its active form in a stepwise manner by enzymes of the DPP IV type in vitro [73]. Given a relative protein content of 1% Api m 5 in native honeybee venom and an amount of 50–140 μg protein delivered per honeybee sting [24], 0.5–1.5 μg DPP IV is injected into the skin per stinging event. However, further studies will be necessary to prove whether the amounts of enzyme injected are actually sufficient to modulate local immune responses in humans.

Hymenoptera Venom Protease Inhibitors

Protease inhibitors are well-represented compounds in Apidae venom [74–77], and recently, they were also found in vespid venom [78]. Various serine protease inhibitors found in snake venom show antifibrinolytic activity [71], while in insects they have been indicated to show multifunctional roles. Api m 6 is a low-molecular-weight allergen in honeybee venom (see Table 3.1) that comprises a trypsin inhibitor-like cysteine-rich domain [74]. It accounts for 0.8–2% of the composition of HBV, and it shows distinct IgE reactivity with patient sera using immunoblot. Next to Api m 6, venom from the honeybee *A. mellifera* contains three serine protease inhibitors (serpins) [46], and in *A. cerana* a Kazal-type serpin (AcKTSPI) with antimicrobial activity could be found [75]. Proteomic analysis of venom from *B. terrestris* revealed three serpins with similarities to serpin 1 and 2 found in *A. mellifera* [23]. A Kunitz-type trypsin inhibitor (Bt-KTI) was also identified that strongly inhibits plasmin, indicating that it acts as an antifibrinolytic agent [76]. The only serine protease inhibitor that has been reported in Hymenoptera other than the Apidae is bicolin [78]. This serpin was characterized from the venom of the aggressive hornet *V. bicolor* that exerted inhibitory activities against trypsin and thrombin, suggesting it might be responsible for the coagulopathy resulted from wasp sting [79].

Two other enzymes, carboxylesterase and serine carboxypeptidase, have been reported in venom from *A. mellifera* [46] and *B. terrestris* [23]. Due to IgE reactivity with patient sera, both compounds in honeybees are known as Api m 8 and Api m 9, respectively (see Table 3.1). Both enzymes could play a role in degradation of insect neurotransmitters [80] and a wide range of proteins [81], respectively.

Hymenoptera Venom Peptides

The dominant compounds in most animal venoms are peptides. They represent a huge source of structurally diverse and biologically active toxins with high potency and selectivity for a range of targets [82]. More specifically, the peptide content of wasp venoms was shown to constitute approximately 70% of the venom toxins [83]. The highest abundant compound in honeybee venom is the 26-amino-acid-long

peptide melittin, constituting ~50% of the venom dry weight. Melittin is a helical amphipathic peptide with hemolyzing activity due to its ability to interact with and disrupt cell membranes [84]. It also causes pain by activating specific receptors in primary nociceptive neurons [85]. In addition, melittin appears to be the main trigger of inflammasome activation [86], which induces a caspase-1-dependent inflammatory response. This is characterized by recruitment of neutrophils to the site of envenomation, which can protect against the damaging effects of envenomation. In addition, melittin is well known for its antimicrobial properties. Its activity may contribute to the recently suggested function of honeybee venom in social immunity of the hive [87, 88]. Indeed, venom peptides are smeared on the body surface of females and on wax combs, which may protect the bees against pathogens attracted to the constant and relatively high temperature and humidity levels maintained in the hive [87]. In bumblebees, bombolitin 1, which shows structural and biological properties similar to melittin, is the most abundant venom compound and has antimicrobial activity [89]. However, a proteomic analysis of bumblebee venom combined with bioinformatics revealed that next to bombolitin 1, no other antimicrobial peptides (AMPs) were present, while honeybee venom was shown to express multiple AMPs. Additionally, the ant *Myrmecia pilosula* contains a venom compound which is related to prepromelittin, the precursor of honeybee melittin, that is also the most abundant venom compound [41]. This peptide named pilosulin-1 is classified as minor allergen Myr p 1, and it displays cytotoxic, hypotensive, histamine-releasing, and antimicrobial activities.

Next to melittin in honeybee venom, mast cell degranulating peptide (MCDP), a neurotoxin which mediates mast cell degranulation at low concentration [90], was found to possess antimicrobial activity [91]. In contrast, the neurotoxic peptide apamin was also suggested to be an antimicrobial agent [87], but recently it was shown to lack antiseptic activity [91]. Recently, the venom of honeybees was shown to contain apidaecin, a small proline-rich peptide that may play important antimicrobial roles [92]. Many hymenopteran species have evolved a venom with antimicrobial properties, which additionally suggests an important function at the organismic level [3]. Indeed, only few pathogens seem to be able to use venom-producing organs of Hymenoptera as a natural route of infection, which shows that microbial diffusions into these organs and from here to the rest of the body are possibly limited by the biochemical venom properties, although also physical barriers may contribute to this observation [3]. In addition, it is remarkable that no significant infectious disease of medical importance has been described to be vectored by hymenopteran stings, while many insects are vectors for microbial pathogens and parasites [3]. In contrast to insect bites, stings are quite rare, which may explain why transmission mechanisms of infectious diseases through venoms have not evolved. Insect venom compounds with antimicrobial properties are not solely restricted to honeybees. Several wasp venom compounds were classified as AMPs with activity against Gram-positive and Gram-negative bacteria [93–95]. In ants on the other hand, pilosulins and ponericins (among others) are venom peptides from the Australian jack jumper ant *M. pilosula* and the neotropical ant *Pachycondyla goeldii*, respectively, which have shown to exhibit broad-spectrum

antimicrobial activity [26]. AMPs in venoms have been widely studied as an alternative to conventional antibiotics, especially for the treatment of drug-resistant infections [96]. Unfortunately, despite the therapeutic efficacy of AMPs, their use is limited due to poor in vivo bioavailability caused by instability, cytotoxicity, and hydrophobicity.

Hymenoptera venoms are known to be strong mediators of mast cell degranulation and histamine release in their stung victims. As mentioned before, honeybee venom contains two peptides with mast cell degranulating (MCD) activities, melittin and MCDP [46, 97]. Vespid venoms often contain multiple mastoparans which are also peptides that have been shown to cause cell lysis at moderate concentrations [47, 49, 94]. Mastoparan peptides are multifunctional toxins that are involved at different levels to produce pain, edema, and inflammation. They activate exocytosis and granule fusion with the plasma membrane, with the consequent release of histamine, proteases, lipid mediators, and cytokines. Venom from the hornet *V. crabro* was found to contain mastoparan C which is known to affect profound histamine release, but was recently found to be a potent antimicrobial by causing generalized membrane interactive and disruptive function [94]. Also several pilosulins from the ant *M. pilosula* were found to exhibit significant and dose-dependent histamine release [41]. The therapeutic use of wasp mastoparans has been investigated since they could be a promising alternative for combating multiple antibiotic-resistant bacteria in clinical practice. A number of strategies for optimizing the potency of mastoparans have been addressed, including structural stabilization and charge modification [98, 99]. However, these studies show that mastoparan activity makes no distinction between bacterial and mammalian membranes. Thus, the development of new strategies to reduce the toxic side effects of mastoparan, thereby improving the feasibility of clinical applications, is required.

Several wasp and ant venoms were shown to contain kinins that are structurally similar to the mammalian pain-producing nonapeptide bradykinin (BK) [100]. When these wasp peptides are injected in humans, they are important mediators of inflammatory responses, potent pain producers, and increased vascular permeability and vasodilatation [101], while in their prey, kinins are used to irreversibly induce paralysis [49]. The giant hunting ant *D. australis* was also shown to possess a peptide in its venom with vasoactive properties like kinin [102].

Many other peptides have been identified in Hymenoptera venom, but their exact functions still remain elusive. In *A. mellifera* venom, the function of two peptides, secapin and tertiapin, is unknown. Secapin possesses no functional domains elucidating its exact function, but due to its moderate/high abundance, it is hypothesized to have a toxic function [103]. Secapin is also a major component of queen bee venom, while it represents at most 1% of the dried venom in workers [104]. Paulistine is another interesting venom peptide, isolated from the wasp *P. paulista* that was demonstrated to cause hyperalgesia by interacting with the receptors of lipid mediators involved in the cyclooxygenase type II pathway [105]. These are only a few examples of the growing list of peptides in hymenopteran venoms that represent the enormous source of chemical diversity. In animal venoms, the evolutionary pressure for improved prey capture and/or defense has resulted in complex

pre-optimized combinatorial peptide libraries with extremely diverse pharmacologies that interact with a wide range of molecular targets. The discovery that these peptides bind to their cognate receptors and ion channels with high affinity and selectivity means that many are now being investigated as sources of lead compounds in therapeutic discovery pipelines [106, 107].

Hymenoptera Venom Proteins with an Unknown Function

For several well-known venom compounds, the function is unclear. Several of these compounds have been characterized as allergens and will be discussed in this section. Antigen 5 (Ag5) is an important and highly abundant venom allergen within the Vespidae and Formicidae (see Table 3.1) [56]. The Ag5 proteins belong to the CAP protein family including cysteine-rich secretory proteins, insect antigen 5 proteins, and plant pathogenesis-related proteins. The Ag5 venom allergens can induce acute hypersensitivity responses in humans. The allergen Vesp ma 5 shares 87%, 72%, 68%, 65%, and 27% sequence similarity with Vesp c 5 from *V. crabro* [108, 109], Dol m 5 from the *D. maculata* [109], Ves v 5 from *V. vulgaris* [110], and Vesp m 5 from *V. maculifrons* [111], respectively, showing that Ag5-like proteins are strongly conserved Hymenoptera allergens. A high degree of cross-reactivity was seen among Ag5 of different genera of hornets and among the genera of yellow jackets, hornets, and paper wasps [108]. These cross-reactivity patterns observed in clinical practice suggest that the commercial extracts of yellow jacket and paper wasp could be prepared with fewer carefully selected species. The Ag5 protein could also be detected in a venom proteome analysis of bumblebees. In honeybee venom, it seems that venom gland tissue of summer honeybee workers does not express this protein, while the Ag5-like protein is secreted by the venom gland of winter bee workers [92]. In contrast to bumblebees and wasps, honeybee workers stay alive during the winter, which may result in differences of the venom composition of summer and winter bees. Unfortunately, the function of the venom Ag5s is unknown, which makes this variation in expression difficult to interpret.

Also the function of the honeybee venom compound icarapin has not been determined. This compound is designated as Api m 10 in the IUIS nomenclature (see Table 3.1) [112]. Recently, nine chimeric transcripts of icarapin were found to be produced by the honeybee venom glands. As neither the function nor the activity of this compound is known, the impact of this heterogeneity on its function remains elusive. However, two highly similar splice variants of icarapin were found to be IgE recognized [113, 114], and they could have the potential to increase the sensitivity of component-resolved diagnosis (see Chap. 5).

In the venom of *A. mellifera* workers and queens, as well as in the venom of *V. vulgaris*, vitellogenin could be identified [18, 115]. Since it could be amplified from venom gland cDNA, it is presumed that it is directly synthesized in the venom gland and secreted into the venom, unlike the transport of vitellogenins into oocytes to serve as food for the developing embryo, where vitellogenins stem from the fat body and are

taken up by receptor-mediated endocytosis [116]. However, honeybee vitellogenin is also thought to act as a multifunctional compound involved in many processes, such as hormone signaling, food-related behavior, immunity, stress resistance, and longevity. Therefore, assumptions for its role as a venom protein remain speculative. Vitellogenin has been added as Api m 12 to the list of honeybee venom allergens [115].

By proteomic approaches, the major royal jelly proteins (MRJPs) 8 and 9 were demonstrated to be components of honeybee venom [84]. They were found to be the most ancient members of the MRJP protein family, which lack the later evolved repetitive regions suggested to have a nutritious function. Therefore, both MRJPs may possess the original but yet unknown pre-royal jelly function [33]. The glycosylated MRJP8 and MRJP9 have IgE-sensitizing potential in HBV-allergic patients beyond CCD reactivity and have to be considered as allergens, which might be potentially important for a fraction of venom-allergic patients [117]. Due to their allergenic properties, MRJP8 and MRJP9 were designated as isoallergens Api m 11.0101 and Api m 11.0201, respectively.

Hymenoptera Venom Trace Compounds

In addition to venoms with a toxic function, the venom may contain lowly and very lowly abundant compounds without toxic activity. These so-called venom trace molecules only have a local function in the venom duct or reservoir (maturation or stabilization of the secretes, protection and recovery of the gland tissue) or are released by leakage of the gland tissue [7]. This categorization has been done for the venom compounds from bumblebee workers and honeybee queens, winter and summer workers, where proteins lacking a signal peptide and having Gene Ontology (GO) terms linked to the intracellular compartments of the secretory pathway were assigned to the group of venom trace molecules [23, 46].

Protein and Peptide Heterogeneity

Besides the identification of proteins and peptides in Hymenoptera venoms, another level of complexity of the venom composition has been revealed. Protein heterogeneity or posttranslational modifications generate different isoforms of several compounds. Protein heterogeneity can be caused by allelic variation at a single gene, by the occurrence of multiple genes encoding highly homologous proteins, or by alternative splicing of a single transcript [56]. In 2001, Kettner and coworkers [118] identified four isoforms of Api m 6 in honeybee venom, only differing in the amino- and carboxy-terminal ends. Using genome-level information, this heterogeneity was later found to be caused by allelic variation [119]. Also, upon 2D gel separation of pure honeybee venom, multiple icarapin protein isoforms were found to exist [103]. In addition, two highly similar icarapin transcripts were found [113], which

differ by only 0.3 kDa in their theoretical molecular weight (MW). A recent study also revealed three alternative splice variants of the honeybee venom compound platelet-derived growth factor 1 (PVF1) [120]. Several wasp venom compounds were also found to show different isoforms [121, 122]. Finally, posttranslational modifications, such as glycosylations and other enzymatic processes, contribute to the venom complexity. SDS-PAGE separation of purified PLA_2 from honeybee venom reveals three protein bands. While the lowest MW band represents non-glycosylated PLA_2, the two higher MW bands correspond to different PLA_2 glycoforms [123, 124]. PLA_2 was also found to occur as a series of different isoforms and/or posttranslationally modified forms in the venom gland of the Brazilian ant species *Tetramorium bicarinatum* [125]. In addition, melittin's inactive precursor promelittin and the complete series of ten conversion intermediates to the mature peptide were detected in crude honeybee venom [61]. This maturation process is executed by DPP IV activity in the venom.

Active Amines

In addition to proteins and peptides, Hymenoptera venoms present biologically active amines, such as acetylcholine, histamine, serotonin, adrenaline, noradrenaline, and dopamine. Serotonin (5-hydroxytryptamine) injection may cause an increase of pain in large animals, lethal vasoconstriction in smaller predators, and neurotoxicity in insects [126]. Histamine, which is the major amine component in several wasp venoms, making up over 90% of all amines [127], produces dilatation and increased permeability of the blood vessel capillaries [128, 129]. Also dopamine and noradrenaline [130] increase the venom distribution by elevating the rate of the heartbeat [13].

Standardization of Hymenoptera Venoms

Allergic reactions to insect stings can be local or systemic. Large local reactions are delayed and prolonged, often causing massive swelling for 5–10 days. Although not life-threatening (unless stung in the throat), they can cause significant morbidity requiring medical treatment. Systemic reactions range from itching, hives, and swelling (on parts of the body distant from the area of the sting) to life-threatening anaphylaxis (throat swelling, trouble breathing, dizziness, or unconsciousness). When insect sting allergy is suspected, diagnostic tests using purified Hymenoptera venoms are needed to confirm the sensitivity and identify the insect species causing the reaction. To provide protection from future stings, venom immunotherapy (VIT) is the treatment of choice. This procedure consists of subcutaneous injections of venom extract in two phases: the incremental and the maintenance dose phase. Different protocols are used for the incremental dose phase, which allow for achieving the maintenance dose phase from within 12 weeks (conventional) to a few days

(rush) or a few hours (ultra-rush). The maintenance dose of 100 μg of venom extract is given every 4–6 weeks usually for 3–5 years [131, 132].

Nowadays, allergen extracts are used for immunotherapy (see component resolved diagnosis, Chap. 5). However, due to great variability in the amounts of individual allergens, these extracts are difficult to standardize. In North America, standardized extracts of purified venom from five common stinging insect species (*A. mellifera*, *Polistes* spp., *D. maculata*, *D. arenaria*, and *Vespula* spp.) are available for the diagnosis and treatment of stinging insect allergy. The term "standardized" indicates that the US Food and Drug Administration (FDA) has established a potency definition for the product and has specified how that potency level will be determined by the manufacturer. Potency labeling for insect venoms is based on the total amount of protein present in the preparation. For each of the individual types of venom, the FDA requires that the reconstituted product contains 100 μg of venom protein per mL of solution. In addition, the FDA has required manufacturers to demonstrate that there is enzymatic activity in the venom preparation. Two types of enzymes are used for this testing: hyaluronidase enzyme must be present and must demonstrate enzymatic activity expressed in units per mL of solution. Typically, the range is from 50 to 130 U/mL. Manufacturers must also demonstrate the presence of phospholipase activity in the preparations, but this is a simple plus/minus test. Next to difficult standardization, lowly abundant but major allergens may be missing in the allergen extracts due to downstream processing [132]. In addition, extracts can be contaminated with allergens from other sources or initiate new IgE specificities [133]. Therefore, the extracts currently used may be insufficient or less safe than natural venom. Recombinant allergens aim to overcome these issues as they can be produced in unlimited amounts, at highly standardized quality and with exact physiochemical and immunological properties. Allergens can be modified to have more favorable characteristics, including reduced IgE reactivity or enhanced immunogenicity [134]. Based on the knowledge of the allergen structures, several approaches have been developed, such as recombinant wild-type allergens, hypo-allergens, T-cell epitope-based vaccines, carrier-bound peptides, genetic vaccination, and gene therapy [133].

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Chapter 4
Clinical Aspects of Hymenoptera Allergy: IgE Response, Stings, and Immunotherapy

Kevin M. White

Introduction

The evaluation of patients for allergy to venom from Hymenoptera is an essential part of the allergist's practice. This chapter will review the types of reactions that occur with sting events, the immune response to stings, and the treatment of Hymenoptera allergy with immunotherapy. Skin and serum testing is covered elsewhere in this volume.

To sting, female Hymenoptera utilize a modified ovipositor that can deliver up to approximately 50 μg (honeybee [1]) or between 10 and 100 ng of venom in the case of fire ants [2]. Types of reactions that occur after a sting include local reactions, large local reactions, systemic reactions (including anaphylaxis), toxic reactions, and unusual reactions. The sting attack rate depends on the insect. Fire ants are notoriously aggressive and exist in high numbers in endemic areas leading to a high sting attack rate. In a group of military medical students training in San Antonio for just 3 weeks, 51% were stung by fire ants [3]. A sample of 183 children from a pediatric clinic in the Southern United States found that 64.4% of children aged 6–10 had detectable fire ant IgE indicating a high sting exposure [4].

K.M. White, M.D.
Department of Allergy, US Air Force, RAF Lakenheath, Brandon, UK
e-mail: kevin.white.7@us.af.mil

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Clinical Reactions to Hymenoptera Stings

A typical ("normal") reaction to a Hymenoptera sting is an inflammatory local reaction characterized by pain, localized edema, and erythema. It normally resolves in 24–48 h. The pain is caused by kinins, edema is caused by vasoactive amines, and enzymes (such as phospholipase and hyaluronidase) cause tissue damage.

A large local reaction (LLR) is largely an IgE-mediated cutaneous reaction, usually greater than 10 cm [5], which may last for several days to a week after the sting event. A simple way to classify a large local reaction is to use the size of the patient's palm as a reference. If the reaction is greater than the size of the patient's palm, it is a large local reaction. Studies demonstrate that depending on the population studied, 20–80% of patients with LLR have venom-specific IgE [6–8]. Despite being sensitized, patients with LLR experience a low risk of future systemic reactions, ranging from 4 to 10%. In one study [7], of 133 patients with LLR, 67 had venom-specific IgE, and only one sensitized patient had a systemic reaction on re-sting. In a study with up to 20 years of follow-up, children with a history of LLR had a risk of systemic reaction of 7% [9]. Interestingly, some populations of patients who have venom-specific IgE but no clinical history of reaction—for example, no history of large local or systemic reaction—have higher rates (up to 15%) of systemic reactions in follow-up than those with LLR [10]. Patients with LLR usually continue to have LLR on sting challenge, rather than reverting to a normal reaction or progressing to a systemic reaction [5, 10].

A systemic reaction is a reaction that includes symptoms away from the sting site. A systemic reaction can involve the skin only, for example, diffuse urticaria after a sting or facial angioedema after a sting on the extremity. Of increased concern are other symptoms typical of anaphylaxis such as wheezing, rhinoconjunctivitis, hypotension, and gastrointestinal cramping. The difference in terminology between "systemic reaction and anaphylaxis" is that a systemic reaction can be cutaneous only—not involving multiple organ systems—but is separate and not solely contiguous with the sting site. In a sample of factory workers in Baltimore, 3% reported having a systemic reaction to flying Hymenoptera [6]. Studies in the pediatric population have reported a prevalence of up to 0.8% [11]. Patients with histories of systemic reactions usually have detectable specific IgE either in skin testing or serum testing, similar to patients with large local reactions. The IgE on the surface of mast cells is bound to high-affinity IgE receptors, and upon binding, venom proteins are cross-linked and activated. This leads to the release of chemical mediators such as histamine, leukotrienes, prostaglandins, and platelet-activating factor, all of which cause the symptoms observed as systemic reactions. It is possible that the difference between patients with specific IgE who react with large local reactions versus systemic reactions is a different threshold of mast cell/basophil activation [5]. In patients who report a systemic reaction to a sting, the risk of having a systemic reaction with each subsequent sting is approximately 50% [12, 13].

This risk can vary however, and can be as low as 25% [14]. Certain predictors help identify patients at higher risk for systemic reactions. Patients with more severe anaphylaxis to stings are at higher risk for a similar reaction on re-sting. Other risk factors for severe sting reactions include mast cell disorders, taking antihypertensive medications, increased age, and preexisting cardiovascular disease. Studies that perform sting challenges have found that in general, the severity of anaphylaxis upon re-sting is not worse than the baseline sting reaction. In a study of 324 patients with a history of systemic reaction from a honeybee or yellow jacket sting, no patients had a more severe reaction on sting challenge [15]. In another study of 175 sting challenges in 111 patients, only one sting challenge reaction was worse than the historical reaction [14].

It has been recognized that patients with systemic mastocytosis are at increased risk for systemic reactions to Hymenoptera stings. In fact, for some patients, venom-induced anaphylaxis is their presenting symptom of indolent systemic mastocytosis [16]. In an observational multicenter study of over 900 patients, elevated baseline mast cell tryptase (BTC) was identified as an independent risk factor for sting reaction severity (a severe reaction being defined as having at least shock or loss of consciousness). Mast cell tryptase levels above 5 μg/L increased the odds ratio for having a severe reaction. A patient with a mast cell tryptase level of 14 μg/L has twice the risk of a severe reaction as one with a tryptase of 5 μg/L [17]. It was later observed in a subset of the same cohort that an elevated BTC also increased the risk of systemic side effects during VIT [18]. The data for the role of BTC in fire ant allergy is limited to case reports [19], although the risk would be expected to be similar.

Cases of refractory anaphylaxis have been reported in patients on beta-blocker medications [20]. Angiotensin-converting enzyme inhibitors (ACE-I) have been identified as a potential risk factor for more severe reactions to field stings [17], but most studies have not shown an increased risk of side effects once the patients were on VIT [18]. A study looking at a population experiencing anaphylaxis from all causes found that an increased risk for severity (in this case, multisystem anaphylaxis) was associated with any antihypertensive medication [21]. Caution may need to be exercised in a patient on any type of antihypertensive, as any medication that lowers blood pressure may reduce the patient's ability to produce vasoconstriction in the face of hypotension. This is further complicated by the fact that patients with cardiovascular disease are at a higher risk for complications from severe anaphylaxis. As the safety of several antihypertensives (including ACE-I) has been demonstrated in patients on venom immunotherapy [22, 23], a venom-allergic patient who needs antihypertensive medication may merit a longer course of immunotherapy.

In an observational study of over 600 patients, increased age was found to be one of the four significant clinical risk factors for more severe anaphylaxis during an index insect sting. The other factors identified were elevated baseline serum tryptase, absence of cutaneous signs in presentation, and more rapid onset of anaphylaxis after sting [24].

Toxic Reactions

A large number of Hymenoptera stings can result in a toxic reaction which is a direct result of the amount of venom injected. The most common example of this is from the Africanized honeybee (*Apis mellifera scutellata*), but it has been reported with vespula [25]. Cases of massive envenomation usually involve hundreds of stings and result in multiorgan dysfunction, including rhabdomyolysis and liver and kidney injury [26, 27].

Unusual Reactions

Many different types of unusual reactions to stinging insects have been reported. Symptoms have been reported in most organ systems and are usually delayed. The most devastating reports are often neurological, involving focal or global deficits. Unusual cutaneous reactions include bullae formation, eosinophilic granulomas, vasculitis, and necrosis. Serum sickness has been reported and its recurrence prevented with immunotherapy [28]. Cases have been described in most organ systems [28–56] and have been the subject of excellent reviews [57, 58]. Selections from these reports are listed in Table 4.1.

Table 4.1 Unusual reactions to Hymenoptera stings

Organ system	Symptoms/syndrome	Insect	Ref.
Nervous	Stroke, ischemic	HB, W	[29, 30]
	Stroke, hemorrhagic	HB	[31–34]
	Ataxia/areflexia/ophthalmoplegia	HB	[57]
	Acute disseminated encephalomyelitis	HB	[35, 36]
	Guillain-Barre	HB	[37]
	Quadriparesis	W	[38]
	Acute polyradiculoneuropathy	W	[39]
	Ataxia and oculopalatal syndrome	HB	[40]
	Epilepsy	IFA	[41]
Cardiovascular	Acute myocardial infarction	HB, W, V	[42–47]
	Arrhythmia	HB	[45]
Skin	Soft tissue necrosis	HB, W	[46, 47]
	Cutaneous vasculitis	IFA	[48]
	Bullae	V	[49]
Renal	Acute kidney injury	W, HB	[50, 51]
	Interstitial nephritis	W	[52]
	Nephrotic syndrome	HB	[53]
	Fanconi syndrome	HB	[54]
Hematologic	Idiopathic thrombocytopenic purpura	HB	[55]
	Henoch-Schönlein purpura	HB	[56]
	Serum sickness	HB, V	[28]

V vespid, *W* wasp, *HB* honey bee

Death

It is estimated that there are 40–50 deaths per year from reactions to Hymenoptera stings, although this data is from the 1970s [59]. When autopsy data is available, the cause of death is usually circulatory collapse or laryngeal edema.

IgE Response to Venom Stings

The immune response to venom is similar to the immune response to other allergens—there are humoral and cellular responses to stings and to venom immunotherapy. Exposure to allergens is through injection into the skin, which presents them to dendritic cells which carry them to regional lymph nodes. In susceptible individuals, a Th2 immune response commences with production of IL-4 and IL-13 which triggers IgE production and thus sensitization. T regulatory cells maintain importance in the decision whether to permit tolerance or an allergic response, even in individuals with immune responses skewed toward Th2. In beekeepers without venom allergy, IL-10 production is linked to regular sting events [60]. The allergist distinguishes patients with a Th2 immune response by the detection of specific IgE to venom proteins (covered in another chapter in this volume).

In populations without history of sting reactions, up to 15% of patients may have detectable specific IgE [6]. Almost 50% of children living in a fire ant endemic area have detectable antibodies (IgE) to fire ants, despite only 2% reporting severe reactions [4]. So detecting venom sensitization is important, though not diagnostic, for venom allergy as the history remains paramount for diagnosis. For this reason, it is not recommended to perform skin or serum testing routinely in patients without a history of a systemic reaction.

After a systemic reaction to a sting, specific IgE may not be detected immediately and may, in some patients, take at least 1 week to become detectable by skin or serum testing [61]. In a study of deliberate sting challenges with Hymenoptera in patients sensitized but not clinically reactive to venom, it was observed that specific IgE only decreases slightly in the hours following a sting. Specific IgE then rises within a week after a sting—often doubling—but may rise as much as 40-fold. IgE levels then fall to baseline by 1 year [62].

Immunotherapy to Hymenoptera

Following the original description of grass injection immunotherapy in 1911 [63], a report of whole body venom extract (WBE) immunotherapy was reported in 1925 to wasp [64]. A case report of immunotherapy with venom (rather than WBE) was reported in 1956 [65]. In 1978, a landmark study was done comparing WBE, venom immunotherapy (VIT), and placebo immunotherapy to yellow jacket and honeybee

hypersensitivity. Patients selected had histories of systemic reactions to stings and positive skin testing to relevant venom. At the end of at least 6 weeks of therapy (and at least 2 weeks at 100 μg of venom or 1:10 w:v WBE), sting challenges were performed. Of the 19 patients treated with VIT, 18 had a sting challenge and one patient developed urticaria. In the WBE group and placebo group, 64 and 58% had a systemic reaction during the sting challenge, a difference that was not statistically significant. This study demonstrated three important facts about venom allergy treatment. First, treatment of flying Hymenoptera with WBE was no more effective than placebo; second, VIT was extremely effective for the treatment of venom allergy; and third, the reaction rate on deliberate sting challenge in susceptible patients is approximately 50% [12]. Due to technical challenges with venom extraction from imported fire ants, only WBE is available for IFA treatment. Nevertheless, it has been demonstrated to be effective when compared to an allergic population who did not receive immunotherapy [66].

Early in the course of immunotherapy, immunological changes are observed. Within the first few injections, a decrease in mast cell/basophil degranulation is observed [67, 68]. This correlates with the rapid protection from anaphylaxis seen in rush immunotherapy. Later on, allergen-specific T regulatory cells develop which suppress Th1 and Th2 cell activity [69]. IgE increases early, then stabilizes, and eventually falls below initial levels. IgG4 serum levels increase as IgE falls but may simply be an epiphenomenon as levels are not predictive of the outcome of sting challenges [70]. After a course of immunotherapy, IgE levels rise after stings (without reactions), which is similar to what happens before patients start immunotherapy [71].

The decision to place a patient on immunotherapy takes into account the patient's history and relevant testing for specific IgE. In general, immunotherapy is recommended for patients with a history of anaphylaxis after a Hymenoptera sting and positive testing for venom-specific IgE. New US recommendations no longer recommend immunotherapy for most patients experiencing a cutaneous-only systemic reaction [72]. This is in line with existing EAACI [73] and BSACI [74] guidelines. Mitigating factors that might influence an allergist to prescribe immunotherapy for a cutaneous reaction might be cardiovascular comorbidities, antihypertensive medications, elevated baseline tryptase, or quality of life concerns (e.g., the potential for unavoidable stings). When selecting the venom for flying Hymenoptera immunotherapy, allergists may either treat the single most likely insect for which there is specific IgE or treatment may be with all venoms to which the patient tests positive. There is significant but variable cross-reactivity among the flying Hymenoptera [75].

Once the decision is made to start immunotherapy, there are several methods of building up to maintenance dosing. These include conventional, cluster, and rush immunotherapy. The difference between these methods is the time it takes to achieve the maintenance dose. Conventional typically takes months, cluster (several shots each visit) takes weeks, and rush immunotherapy takes 1–3 days to reach maintenance. Which method to use is often dependent on the risk of being stung versus the risk of treatment (which is discussed further below). A patient with a history of severe hypotension with vespid stings might be best served by ultra-rush immunotherapy if started in the summer when there is high risk of being stung but offered conventional

if started in the middle of winter when the risk of being stung is low. Patient preference may also play a role—for example, avoiding missed work or school may influence a decision to offer rapid buildup. Institutional patterns may also play a role, as centers of excellence with rush or ultra-rush immunotherapy may be more likely to offer a protocol with which they have extensive expertise. Venom immunotherapy is always done with injections, although sublingual immunotherapy has been studied with large local reactions and found to reduce their size [76]. See Table 4.2 for examples of published protocols for Hymenoptera immunotherapy.

Table 4.2 Recommended venom immunotherapy schedules

Flying Hymenoptera								
Conventional [95]			Cluster 30 min intervals [96]			Rush [79]		
Week	μg/mL	Vol(mL)	Week	μg/mL	Vol(mL)	Day	μg/mL	Vol
1	1	0.05	1	0.01	0.1	1	0.1	0.1
2	1	0.1		0.1	0.1		1	0.1
3	1	0.2		1	0.1		10	0.1
4	1	0.4	2	1	0.1		100	0.1
5	10	0.05		1	0.5		100	0.2
6	10	0.1		10	0.1		100	0.4
7	10	0.2	3	10	0.1		100	0.8
8	10	0.4		10	0.5	2	100	1.0
9	100	0.05		10	1.0			
10	100	0.1	4	100	0.1			
11	100	0.2		100	0.2			
12	100	0.4	5	100	0.2			
13	100	0.6		100	0.3			
14	100	0.8	6	100	0.3			
15	100	1.0		100	0.3			
			7	100	0.4			
				100	0.4			
			8	100	0.5			
				100	0.5			
			9	100	1.0			
Imported fire ant schedules								
Conventional [75]			**Rush (30 min intervals) [78]**					
Week	**wt:vol**	**Vol**	**Day**	**wt:vol**	**Vol**			
1	1:100,000	0.05	1	1:100,000	0.3			
2	1:100,000	0.15		1:10,000	0.1			
3	1:100,000	0.25		1:10,000	0.3			
4	1:100,000	0.5		1:1,000	0.05			
5	1:10,000	0.05		1:1,000	0.15			
6	1:10,000	0.1		1:1,000	0.3			
7	1:10,000	0.2		1:100[a]	0.05			

(continued)

Table 4.2 (continued)

Flying Hymenoptera								
Conventional [95]			Cluster 30 min intervals [96]			Rush [79]		
Week	μg/mL	Vol(mL)	Week	μg/mL	Vol(mL)	Day	μg/mL	Vol
8	1:10,000	0.3		1:100[a]	0.1			
9	1:10,000	0.4		1:100[a]	0.2			
10	1:10,000	0.5		1:100[a]	0.3			
11	1:1000	0.05	8	1:100	0.5			
12	1:1000	0.1						
13	1:1000	0.2						
14	1:1000	0.3						
15	1:1000	0.4						
16	1:1000	0.5						
17	1:100	0.05						
18	1:100	0.07						
19	1:100	0.1						
20	1:100	0.15						
21	1:100	0.2						
22	1:100	0.25						
23	1:100	0.4						
24	1:100	0.5						

[a]Doses are 60 min apart

As with flying Hymenoptera, rush immunotherapy to fire ant whole body extract has been shown to be safe and effective, whether studied as a 2-day protocol [77] or an updated 1-day protocol [78]. Suggested rush protocols for both flying Hymenoptera and fire ant are included in Table 4.2. Many protocols have demonstrated similar systemic reaction rates to conventional venom immunotherapy without the use of premedication such as oral steroids and antihistamines [67, 79].

The maintenance dose of flying Hymenoptera is typically 100 μg of venom injected subcutaneously monthly for each species of venom administered. There is data that 50 μg might be protective in some cases [13]. Fire ant WBE dosing, since it is not standardized, is typically 0.5 mL of a 1:100 wt/vol solution [66, 80] which is used by the majority of allergists in a national survey, although some allergists used a 1:10 wt/vol solution [81]. That maintenance dose has been shown to protect against fire ant sting challenges in several clinical studies with rush immunotherapy [77, 78]. Patients who are not protected by standard doses of Hymenoptera venom, e.g., 100 μg monthly, may be protected by higher doses of venom immunotherapy [82].

Once at a monthly maintenance immunotherapy for 1 year, there is data to suggest that patients may not need monthly maintenance dosing to remain protected against anaphylaxis. Thirty patients on monthly yellow jacket or honeybee VIT tolerated sting challenges annually for 2 years while receiving monthly immunotherapy. Eighty percent of the patients originally presented with hypotension or respiratory distress. They were then given VIT 6 weeks apart for 6–9 months before

being sting challenged again. One patient was unavailable for sting challenge, and one patient had a subjective reaction manifested by anxiety; efficacy was 97% [83]. A study in 2001 extended the interval to 12 weeks as early as 8 months on VIT. Patients were on maintenance VIT for 4 months and then received a dose at 6 weeks, then 8 weeks, and 12 weeks thereafter. Most patients were on honeybee VIT. Of 160 patients, 36 experienced field stings while on 3-month intervals. One patient had a mild cutaneous reaction and one had a subjective reaction. Forty-six patients had sting challenges with two patients having mild systemic reactions (Grades I and II, respectively). Again, the efficacy was similar to studies with 4-week maintenance VIT [84]. This concept has also been demonstrated with preliminary data extending fire ant WBE maintenance to 12-week intervals [85].

Venom immunotherapy is continued normally for 5 years of maintenance immunotherapy or when skin testing to the venom becomes negative. The severity of the original reaction dictates the length of immunotherapy. If the reaction were mild, for example, a systemic cutaneous reaction in an adult, then 5 years of immunotherapy would greatly reduce the risk of subsequent anaphylaxis after stopping treatment. If the reaction was severe and caused refractory hypotension, the treating physician and patient may not tolerate any recurrence of risk after stopping immunotherapy, and the patient may remain on lifelong therapy. This topic is discussed in more detail in another chapter in this volume.

Side effects from immunotherapy include local and systemic reactions. Large local reactions to immunotherapy mimic the potential reactions to venom stings themselves but are rare and usually do not impede the progress toward maintenance. Systemic reaction rates can be described in terms of rate per injection or the overall rate for patients over the course of their immunotherapy. When counseling patients about the risk of venom immunotherapy, the per-patient rate is more practical to review than the per-injection rate. In the original study of VIT which used a modified cluster protocol (up to 10 μg venom on the first day of treatment), 1 out of 19 patients (5%) had a systemic reaction during 2–3 months of therapy [12]. A large multicenter European prospective study found that 20% of patients on VIT had a systemic reaction during 1–2 years of treatment, or 1.2% of injections. Most (two thirds) of the reactions were mild; ultimately 6% of patients receiving VIT had systemic side effects requiring treatment. The study found that systemic reactions were more frequent in the buildup phase (1.9% vs. 0.5% of injections) and more likely to occur with rush immunotherapy [86]. A multicenter US-based observational study of 1410 patients on Hymenoptera immunotherapy reported an overall systemic reaction rate of 12% with most (60%) being mild [87]. Risk factors for systemic reactions included buildup phase (between 1 and 50 μg) and honeybee immunotherapy. Patients with mastocytosis treated with venom immunotherapy appear to have a higher risk for systemic reactions to VIT during the buildup phase and receive less protection than expected (perhaps 75%) [88].

Some patients who have systemic reactions to conventional immunotherapy are able to achieve maintenance dosing through rush immunotherapy. Nine patients allergic to flying Hymenoptera (mainly honeybee) who did not tolerate VIT because of recurrent systemic reactions underwent a rush protocol using 58.55 μg on day 1

and 100 μg on day 2. Seven of the patients successfully achieved 100 μg maintenance dosing, although five of them required modification of the protocol, either slowing it down or using loratadine as premedication [89]. Omalizumab can protect against systemic reactions to venom immunotherapy. There have been case reports of patients who have had reactions to VIT prevented with omalizumab in flying Hymenoptera [90–92] and IFA [93]. Failure of omalizumab has also been reported [94]. The use of omalizumab has also been reported successful in cases of patients with Hymenoptera allergy and mast cell disorders. Dosing normally is started 12 weeks prior to VIT.

In conclusion, Hymenoptera stings can cause a variety of clinical reactions including toxic and unusual syndromes which the allergist is uniquely suited to evaluate. Patients who are sensitized to venom may be asymptomatic, have large local reactions, or may experience anaphylaxis. For the patients with histories of anaphylaxis, there is substantial data they can be protected with venom immunotherapy (flying Hymenoptera) or whole body extract immunotherapy (fire ant). Rapid protocols exist that can achieve maintenance dosing in as little as 2 days. Side effects do not normally impede achievement of a protective maintenance dose, but adjuncts exist (e.g., adjusted maintenance dose or omalizumab) for challenging cases. The practicing allergist thus has a wealth of tools available to evaluate and treat patients with venom allergy.

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Chapter 5
Diagnostic In Vivo and In Vitro Methods in Insect Allergy

Robert G. Hamilton

Abbreviations

BAT	Basophil activation test
BHR	Basophil histamine release test
CCD	Carbohydrate cross-reactive determinant
ELISA	Enzyme-linked immunosorbent assay
HBV	Honeybee venom
ID	Intradermal
PWV	*Polistes* wasp venom
ST	Skin test
YJV	Yellow jacket venom

The diagnosis of Hymenoptera venom allergy begins with collecting evidence of an allergic reaction to an insect sting through a detailed patient history and physical examination [1]. The physician uses this information to determine a pretest likelihood of a sting-associated allergic response. The majority of insect stings produce a localized redness, swelling, itching, and pain that occur around the site of the sting. In cases of large local reactions, erythematous swelling can be extensive and may persist for several days or more. Other factors that need to be considered in the diagnostic process include the symptom characteristics (e.g., urticaria and angioedema, bronchospasm, airway edema, and/or hypotension and severity of the reaction and duration and delay time of the appearance of the reaction from the sting event), identification of the culprit, number of stings, location of sting on the body,

R.G. Hamilton, Ph.D., DABMLI, FAAAAI
Division of Allergy and Clinical Immunology, Departments of Medicine and Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Johns Hopkins Asthma and Allergy Center, Johns Hopkins Dermatology, Allergy and Clinical Immunology (DACI) Reference Laboratory,
Room 1A20, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, USA
e-mail: rhamilt2@jhmi.edu

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experience with past stings, exposure risk (e.g., geography, hobbies [gardening, beekeeping]), the patient's atopic factors (personal, family, age), and use of medications (antihistamine, beta-adrenergic blocking agents) [2, 3]. Once a strong suspicion has been derived from the patient's history that an immediate-type hypersensitivity reaction has occurred as a result of an insect sting, confirmation of sensitization is routinely obtained. This involves the detection of venom-specific IgE antibody by in vivo and in vitro methods. The presence of detectable venom-specific IgE antibodies in the skin or blood of the patient is considered only a risk factor for but not a definitive indicator of insect allergy. There must be a concordant positive history of a sting-associated allergic reaction together with a positive IgE antibody response to make the diagnosis. In cases where the confirmatory skin or serology test results are discordant with the history, the basophil activation test can provide clarity. Definitive evidence for insect allergy can ultimately only be derived from an intentional sting challenge (direct provocation test) [4]. Since Hymenoptera sting challenges are logistically difficult to perform in a clinician's office and associated with a risk for possible systemic reactions, they are rarely performed except in cases of consented research studies where the measured endpoint requires definitive documentation of an immediate reaction.

In Vivo Tests for Confirmation of Sensitization

Skin tests: Skin testing (ST) is the principal diagnostic confirmatory test that is used to document sensitization to venoms from three primary Hymenoptera families: Vespidae (*Vespula*, *Dolichovespula*, and *Polistes*), Apidae (*Apis* and *Bombus*), and Formicidae (*Solenopsis*) [1]. Intradermal skin tests are considered the most diagnostically sensitive and biologically relevant, but they are less diagnostically specific in comparison with an epicutaneous skin test or IgE antivenom serology. Intradermal (ID) injections of the complete set of five medically important Hymenoptera venoms are placed on the volar aspect of the arm or back at concentrations increasing from 0.001 to 1.0 μg/mL. At venom concentrations greater than 1 μg/mL, a high percentage of false-positive irritant reactions are known to occur [5, 6]. Pre-skin test administration of certain drugs such as antihistamines compromise the accuracy of the skin test, and thus the pharmacologically treated patient is required to wait a sufficient period of time before receiving a skin test.

The composition of diagnostic Hymenoptera venom extracts that are used in skin testing is complex [7]. The historical use of whole-body extracts that contain little or no venom was shown to be unable to distinguish allergic from nonallergic individuals. Thus, whole-body extracts have been abandoned, except for fire ant, in favor of the use of extracted purified venoms [1]. The heterogeneous diagnostic venom extracts contain a spectrum of allergenic (Table 5.1) and nonallergenic proteins as well as vasoactive amines (histamine, dopamine, and norepinephrine), acetylcholine, and kinins that can induce burning, itching, and pain localized to the sting site. Removal of the small amines by dialysis of the venom permits true

Table 5.1 Hymenoptera venom allergens

Allergen	Molecular weight (kDa)	Biological function/common name
Apinae		
Apis mellifera (honeybee)		
Api m 1	15	Phospholipase A2
Api m 2	41	Hyaluronidase
Api m 3	49	Acid phosphatase prostatic
Api m 4	3	Melittin
Api m 5	100	Allergen C/DPP IV
Api m 6	8	Protease inhibitor
Api m 7	39	Protease
Api m 8	70	Carboxylesterase
Api m 9	60	Carboxypeptidase
Api m 10	55	CRP/carapin
Api m 11	60–65	MRJP 8–9
Api m 12	200	Vitellogenin
Bombinae		
Bombus pennsylvanicus/terrestris (bumblebee)		
Bom p 1-Bom t 1	16	Phospholipase A2
Bom p 4-Bom t 4	27	Protease
Vespoidea/Vespinae		
Vespula vulgaris (yellow jacket)		
Ves v 1	35	Phospholipase A1
Ves v 2.0101	45	Hyaluronidase
Ves v 3	100	DPP IV
Ves v 5	25	Antigen 5
Ves v 6	200	Vitellogenin
Dolichovespula maculata/arenaria (hornets)		
Dol m 1	34	Phospholipase A1
Dol m 2	42	Hyaluronidase
Dol m 5-Dol a 5	23	Antigen 5
Polistinae, *Polistes annularis/exclamans* (paper wasp)		
Pol a 1	34	Phospholipase A1
Pol a 2	38	Hyaluronidase
Pol e 4	?	Protease
Pol a 5-Pol e 5-Pol d 5	23	Antigen 5
Formicidae		
Solenopsis invicta (red imported fire ant)		
Sol i 1	37	Phospholipase A1
Sol i 2	26/dimer	Unknown
Sol i 3	24	Antigen 5
Sol i 4	13.3	Unknown

positive intradermal skin reactions to be detected at concentrations above 1 μg/mL [8]. Simultaneous administration of a negative control diluent (human serum albumin in saline) and a positive control histamine allows the documentation of dermatographism and pre-skin test antihistamine interference, respectively. The ID-ST technique that is used in clinical practice is not standardized among all clinicians. Different volumes of venom are reportedly administered intradermally into the skin (e.g., 0.02–0.05 mL or a volume sufficient to raise a 3, 4, or 5 mm bleb). Moreover, the minimum time of reading and criterion that is used to define a positive intradermal skin test with venom can vary from a wheal 3 mm greater than the negative control plus surrounding erythema to 5–10 mm wheal and greater than 10 mm erythema to a 3 or 5 mm wheal regardless of erythema at 15 or 20 min. For individuals with a history of extremely severe reactions, a safer form of epicutaneous skin testing can be performed with venom that is typically at 1 μg/mL. Even the result of an epicutaneous skin test, despite its inherent simplicity, can be altered by interphysician variation in scoring and interpretation [9]. Moreover, there are currently no certification, systematic proficiency, or quality control programs available to monitor the extent of intradermal or puncture skin test reproducibility and qualify skin testers.

The majority of patients with a convincing history of insect sting allergy have a positive skin test. However, up to one third of individuals with a positive history of a systemic reaction can have a negative skin test, and a subset of these can experience a systemic reaction to a subsequent sting [8, 10]. The causes of this discordance are not known, but they are believed to stem from either a refractory period of anergy in the skin, days or weeks after a sting, missing or labile allergens in the venom extract, a true loss of sensitivity resulting from a distant history of a sting reaction, or a non-IgE-mediated reaction possibly that may involve subclinical mastocytosis [11]. Those insect allergic patients with a negative venom skin test and a convincing history of anaphylaxis should be re-skin tested and further evaluated by serology since one third of these can have venom-specific IgE antibodies that are detectable in their serum or a positive repeat skin test [8].

Sting challenge provocation test: The Hymenoptera sting challenge with a living insect has been considered a definitive reference method for verifying insect sting allergy and clarifying asymptomatic sensitization [12]. Stinging with the appropriate offending insect, providing it is known, permits documentation of the patient's unique sensitization profile, and thus it permits optimal venom selection for administration under a physician's supervision. It also provides an objective measure of any localized or systemic response. There are, however, significant challenges such as obtaining the appropriate insect for the patient's scheduled visit, insuring reproducible quantities of venom are injected by the insect and addressing concerns about the lack of reproducibility of systemic reactions and the potential for a severe reaction. These concerns have minimized the general use of the Hymenoptera sting challenge as a routine diagnostic procedure. Finally, the outcome of a single diagnostic sting challenge needs to be interpreted with caution because of its reported poor reproducibility and lack of predictive value for the occurrence or severity of the patient's anaphylaxis risk with a subsequent sting [13, 14].

In Vitro Tests for Confirmation of Sensitization

Serological venom-specific IgE antibody analyses: Venom-specific IgE antibody serology is currently considered complementary to the epicutaneous venom skin test. It is particularly useful in cases where the patient is dermatographic on a medication that can compromise the skin test result, or they are skin test negative with a strong positive history of a life-threatening reaction. IgE antibody assay technology has improved greatly over the years. Enhancements have included increased precision due to automation and improved analytical sensitivity with the availability of higher capacity allergosorbent materials that insure molar excess of immobilized or liquid phase allergenic proteins [15]. The venom extracts used in serological assays have become more highly quality controlled and reproducible, and there are more avid and specific labeled polyclonal and monoclonal antihuman IgE detection reagents that use safer non-isotopic labels. The calibration schemes used by the present-day autoanalyzers are traceable to the World Health Organization's third total serum IgE reference preparation [16]. There are independent and more extensive interlaboratory proficiency and inter-assay quality control programs [17]. The data analysis algorithms are more precise and quantitative. Finally, and possibly most important, there is a panel of newly available recombinant venom allergen molecules that will enhance the analytical sensitivity and specificity of the current venom extract-based-specific IgE antibody serological assays (expanded upon below).

These improvements have been incorporated in autoanalyzers produced by Thermo Fisher Scientific (ImmunoCAP) and Siemens Healthcare (Immulite), which dominate clinical laboratory use throughout the Western world [18]. Both are state-of-the-art assay methods that permit quantitative measures of venom-specific IgE detection in serum. The ImmunoCAP uses venom allergens which are covalently bound to an activated hydrophilic polymer solid phase to bind IgE antibody from serum. In contrast, the Immulite uses venom allergens that are covalently attached to a soluble biotinylated polylysine polymer that remains in a fluid phase as it interacts with IgE antibody and then binds to a streptavidin-covered polystyrene ball. While both autoanalyzers have similar specifications in their use of enzyme-labeled anti-IgE detection antibodies and reported minimum detectable concentration, they tend to detect different quantitative levels of IgE antibody. These quantitative differences are believed to stem from differences in the allergen compositions on their allergosorbents and the use of different calibration algorithms [18–20].

Since 1978, a venom-specific IgE radioallergosorbent test has been performed in one clinical immunology laboratory which has been used by some as a reference assay [21]. Due to interest in a possible differential assay sensitivity of this RAST with the commercially available ImmunoCAP, comparative performance of the two assays was directly compared using sera from patients with a positive clinical history of Hymenoptera venom sensitivity and a positive venom-specific IgE antibody serology. The study identified a strong, positive correlation between the venom-specific IgE antibody levels produced with both assay methods ($r^2 = 0.92$, $p < 0.001$,

$Y = 0.94 \times 10^{-5}$, $n = 294$) [22, 23]. Only 6.8% (19/294) of the sera were negative (<0.35 kUa/L) in the ImmunoCAP and positive for venom-specific IgE antibody by RAST. If the currently accepted ImmunoCAP analytical sensitivity threshold of 0.1 kUa/L had been used, the agreement would have been even better. The conclusion is that for more than 95% of sera, the ImmunoCAP system and the JHU RAST produce diagnostically comparable results. Comparable performance data is not available for the Immulite system. Finally, the performance of serological assays for IgE antibody is not interfered with by patient premedication with antihistamines or steroids, and IgE antibody remains stable in serum when stored frozen at least 5 years prior to analysis [24].

Basophil-based IgE antibody sensitization tests: Since basophils and mast cells are key effector cells involved in immediate-type hypersensitivity reactions, the in vitro basophil activation test (BAT) and basophil mediator (histamine) release (BHR) assay have been considered surrogates that can reflect in vivo activation of mast cells by allergen [25]. Activation of the basophil by allergen as it cross-links surface FcεRI-bound IgE antibody reflects a more complex functional response that cannot be obtained with serological assays which can only measure the quantity of allergen-specific IgE antibody circulating in the blood. CD63 and CD203c are basophil membrane proteins that are localized to the same secretory granules which contain histamine [26, 27]. CD63 is also present on platelets that sometimes adhere to basophils and can lead to erroneous activation results. The extent of basophil activation by allergen-induced cross-linking can be monitored by either quantifying the amount of cysteinyl leukotriene or histamine released into cell supernatants by ELISA or fluoro-spectroscopic assays or by measuring the magnitude of upregulation or increase in expression of CD63 or CD203c using monoclonal antibody-based flow cytometry. Both the level of mediators released and cell surface marker density expressed correlate with blood basophil and tissue mast cell activation [28]. Hoffmann et al. [25] in their position paper make the case that the BAT may be more amenable to clinical evaluation of patients than the BHR assay. The measurement of histamine and cysteinyl leukotriene can be technically more challenging, while fluorocytometric analysis for quantification of cell surface marker expression can be performed in both clinical and research laboratories equipped with a flow cytometer. A graphic illustration of the gating strategy involving two-color flow cytometric staining of IgE and CD63 on basophils which were unstimulated or stimulated with anti-FcεR1 (positive control) or native wasp venom is presented in Fig. 5.1 [29]. Recombinant Ves v 1, 2, 3, and 5 have also been used to show that venom allergenic molecules can fully activate basophils from most venom allergic subjects and that allergen-induced CD63 expression can clearly distinguish allergic from nonallergic subjects.

The BAT assay has unique requirements for blood collection in heparin (not EDTA or acid citrate dextrose), and individuals should avoid taking steroids or cyclosporine A before providing blood. Interleukin-3 which is often added to the BAT analysis enhances allergen-specific CD63 upregulation, but IL-3's impact on assay accuracy is a possible concern. Isolated cells are incubated with positive (anti-

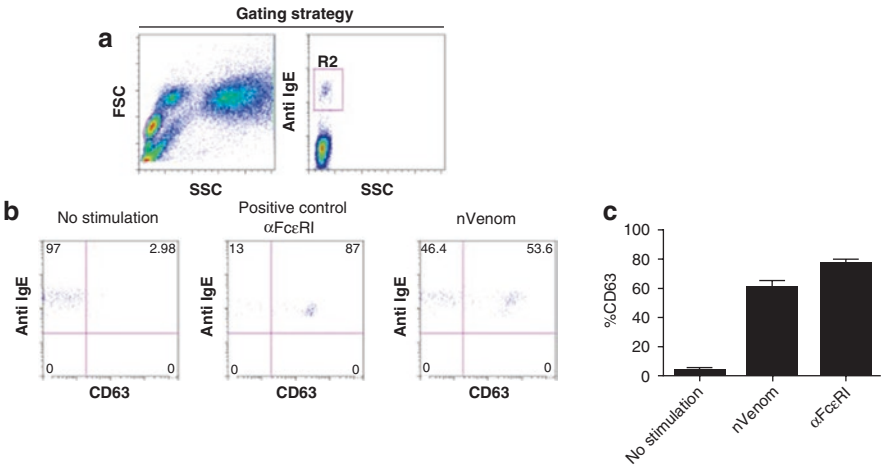


Fig. 5.1 Illustration of the gating strategy and data output of the basophil activation test. Panel **a** illustrates the identification of basophils first based on their size and density which defines the measured magnitude of the forward and side scatter (FCS and SSC, respectively). Basophils are further detected by gating on the cell population that has surface IgE that is bound by fluorophore-conjugated anti-IgE. Panel **b** illustrates two-color flow cytometric staining dot plots of IgE and CD63 (using fluorophore-conjugated anti-IgE and anti-CD63) of cell preparations that have been stimulated with buffer (no stimulation) anti-FcεR1 (positive control) or wasp venom. Panel **c** presents the percentage of CD63 expression (mean ± SD) on basophils in the absence of stimulation (negative control), or stimulated with anti-FcεR1 (positive control) or wasp venom (test condition) (Reproduced with permission from [29])

human IgE), negative (saline) controls, and several concentrations of venom antigen to determine optimal basophil sensitivity (50% maximum response) or reactivity (maximum response) [25]. The concentrations of venom used in the basophil assays range from pg/mL to μg/mL, and each venom preparation needs to be validated by itself so it is shown to be nontoxic to basophils and that it does not elicit nonspecific activation. The same characterized venom preparations should be employed if the BAT is being repetitively used to monitor a given patient longitudinally over their course of venom immunotherapy or over a period of allergy resolution involving insect sting avoidance.

The BAT test has been particularly useful in clarifying the diagnostic status of patients suspected of having Hymenoptera venom allergy who have equivocal or negative venom skin test and IgE antivenom serology test results and in determining if dual yellow jacket and honeybee venom skin test or serology positivity is true dual sensitization or due to venom carbohydrate cross-reactivity. Ebo et al. [30] showed that the BAT was able to confirm venom sensitization in 6% of their cases with a positive clinical history but a negative skin test and IgE antibody serology. Furthermore, the BAT results were useful in clarifying sensitization to single or dual venoms in 24% of their study population with IgE to both yellow jacket and honeybee venom. Of their 118 study patients, 17% could not be tested in the BAT as they

were nonresponders who exhibited no CD63 upregulation after stimulation with the anti-IgE positive control. Other studies corroborate the utility and limitations of BAT in diagnostically assessing Hymenoptera venom sensitization [31, 32].

When considering routine diagnostic testing, the BAT has some strengths and limitations. Its strength resides in relative simplicity and the fact that it is the only in vitro assay that reflects functional activity rather than just the level of circulating immunoreactive IgE antibody. The BAT, however, is not useful for patients who are non-releasers which represents 10–15% of individuals. It is considered less sensitive than the intradermal skin test. Venom preparations are rarely standardized for use in basophil assays, and the optimal venom concentration appears to be basophil (patient) dependent. The BAT assay requires processing blood within 24 h of collection and the use of IL-3 priming which can produce false-positive results.

Unique Diagnostic Considerations of Stinging Formicidae (Ants)

While approximately 8800 species of the Formicidae family are found worldwide, only few ant species induce allergic responses [33]. The likelihood of experiencing a systemic reaction to an ant sting depends on the probability of human contact, extent of venom allergenicity, local distribution of the ant colony, and degree of aggression manifested by the species. Hoffman [34] reported that each *S. invicta* ant sting can deliver piperidine alkaloids that cause localized sterile pustules and between 10 to 100 ng of biologically active peptides and proteins in 0.04–0.11 μL of venom. A number of these proteins can elicit sensitization and allergic reactions in sensitized individuals.

The diagnosis of stinging ant allergy follows an analogous algorithm to the other Hymenoptera which involves an initial detailed clinical history that if positive is followed by confirmation of sensitization with IgE antibody detection. The diagnostic golden rule still applies that presence of fire ant venom-specific IgE antibody alone does not in and of itself predict the presence of systemic reactivity. Thus, the evaluation of *localized* reactions to ant stings is typically restricted to documentation of the patient's clinical signs and symptoms. The exception may be occupationally exposed individuals who are at risk for repetitive insect stings (e.g., honeybee keepers, gardeners) [35, 36]. In contrast, *systemic* reactions require confirmation of sensitization by detection of IgE antibodies. Skin testing is the preferred IgE antibody confirmatory test. In cases where a patient has a negative skin test but a definitive positive history of a systemic reaction, serological evaluation should be additionally performed. If the serology is also negative, repeat skin testing with another manufacturer's reagent is recommended [8, 37].

Currently, fire ant whole-body extracts (1:10 weight/volume stock solutions) are available for diagnostic skin testing. Due to the large variability in their potency, Sol i 2 and Sol i 3 content of these extracts is routinely documented by American

manufacturers. Whole-body extracts are typically diluted to 1:100 w/v for use in initial epicutaneous (prick/puncture) skin testing. If the puncture skin test is negative, intradermal skin testing is performed, starting at a 1:1,000,000 w/v and increasing tenfold to 1:500 w/v. A wheal at least 3 mm greater than the saline control at a 1:500 w/v or lower concentration is considered evidence of venom-specific IgE presence [38]. Diagnostic sensitivity of skin testing with fire ant whole-body extracts is reportedly 76–94%, with about 20% of patients who have a negative skin test generating a positive IgE antibody serology. In patients taking antihistamines, high-dose beta-adrenergic blocking medications or those with dermographism, IgE antibody serology is considered the initial confirmatory test of choice. Commercial serological assays for fire ant venom-specific IgE use immobilized whole-body fire ant extracts as their allergosorbent. Isolated fire ant venoms are in limited use by some laboratories and research settings, and they have been shown to provide superior confirmatory test diagnostic performance when used in both skin testing and serological detection of IgE antibody [39–41]. The diagnostic sensitivity of the commercially available fire ant-specific IgE assays is reportedly comparable to intradermal skin tests that use whole-body extracts as their allergen source [41].

Impact of Molecular Allergology on Insect Venom Allergy Diagnosis

Hymenoptera venoms used in in vivo or in vitro diagnostic tests are complex mixtures of proteins, many with enzymatic activity. Table 5.1 presents the principal allergenic venom molecules for the medically important Hymenoptera that have been identified. Sophisticated recombinant methods involving bacteria and more recently eukaryotic cells of insect origin have been used to generate immunoreactive venom allergens that possess proper folding and glycosylation [42]. The physicochemical characteristics, 3D structures, and biological properties of these allergenic venom molecules are discussed in detail elsewhere [43, 44].

Flying Hymenoptera: The best characterized molecular allergens from honeybee venom (HBV) are Api m 1 (phospholipase A2), Api m 2 (hyaluronidase), and Api m 4 (melittin). The most well-studied yellow jacket venom (YJV) allergens are Ves v 1 (phospholipase A1), Ves v 2.0101 (hyaluronidase), and Ves v 5 (antigen 5). Of these, Api m 1, Ves v 1, and Ves v 5 and Pol d 5 for *Polistes* wasp are the only specificities presently available for molecular diagnostic testing of patients. Multiple studies have shown that 92–96% of YJV allergic individuals can be effectively identified using Ves v 1 and Ves v 5 [20, 44, 45]. In contrast, only 58–80% of HBV-sensitized patients could be identified by the serological detection of IgE anti-Api m 1 alone. When Api m 2, 3, 4, 5, and 10 allergen specificities were added in a research study to the IgE antibody analysis, 95% of the 144 patient with a confirmed HBV allergy were identified as positive, with 74% of these having an IgE antibody response to more than one allergen specificity [46]. In another study, Selb et al. [19]

reported that the Immulite displayed a significantly higher diagnostic sensitivity in comparison to the ImmunoCAP using rApi m 1 (71% vs. 88%) and rVes v 5 (82% vs. 93%) while displaying comparable diagnostic specificity for both allergens (94–98%). Schrautzer et al. [20] confirmed these observations by demonstrating a 1.4-fold higher diagnostic sensitivity with the Immulite rApi m 1 as compared to the CAP rApi m 1, with an equivalent diagnostic specificity for both systems. Using the Immulite system, Michel et al. [47] detected no differences in the IgE anti-recombinant YJV (Ves v 1 and 5) and HBV (Api m 1–4 and 10) component allergen profiles among venom allergic patients with and without elevated baseline tryptase or mastocytosis. This supports the conclusion that the specificity of the IgE antivenom response does not appear to be a major factor in determining the reaction risk, severity of a reaction, or the outcome of venom immunotherapy, even in individuals with an elevated baseline tryptase level. As more venom allergenic molecules become available for clinical testing, the relative diagnostic performance of the two autoanalyzers will need to be reexamined.

Molecular venom allergens may have possibly their most important future application in clarifying whether venom allergic patients with IgE antibody positivity for two or more distinct venoms (YJV, HBV, and/or *Polistes* wasp venom [PWV]) reflects true multiple venom sensitization or whether the observed multiple positivity is due to either clinically irrelevant cross-reactive carbohydrate-specific IgE antibodies or common protein epitopes of homologous venom antigens [48]. The relevant cross-reactive carbohydrate determinant (CCD) in insect venoms involves an alpha 1,3-linked fucose residue that is attached to the innermost N-acetylglucosamine carbohydrate on venom proteins [7]. IgE antibodies to this carbohydrate structure that can be detected using immobilized bromelain, rape pollen, horseradish peroxidase, and MUXF as sources of CCDs are reportedly responsible for up to 75% of YJV and HBV double sensitization [49]. Research CCD-free recombinant venom allergens such as recombinant Api m 1 can be useful in excluding CCD cross-reactivity responses [50].

The other source of cross-reactivity among the venoms involves the common protein epitopes on homologous allergenic proteins present in several venoms. For YJV and HBV, currently known common proteins include hyaluronidases (Api m 2, Ves v 2), dipeptidyl peptidases (Api m 5, Ves v 3), and the vitellogenins (Api m 12, Ves v 6) [51, 52]. IgE antibody cross-reactivity between YJV and PWV is independent of CCD and can occur as a result of common epitopes on homologous antigens: Antigen 5 (Ves v 5, Pol d 5) and phospholipase A 1 (Ves v 1 and Pol d 1) [53]. Using these PWV and YJV molecules, the most probable sensitizing insect could be defined in ~70% of double positive patients, and in the remainder, double sensitization could not be excluded. Caruso et al. [54] reported that specific IgE antibody measurements against rVes v 5 and rPol d 5 alone were able to aid in differentiating sensitivity against *Vespula* and *Polistes* venoms in some patients. However, Savi et al. [55] show the inability of these molecular allergen analyses to definitively identify the culprit sensitizing venom(s) in dual sensitization cases. They concluded that the competitive venom inhibition analysis remains the sole serological assay at

present which can distinguish true single from dual sensitization in patients who have positive IgE antibody tests to both *Vespula* and *Polistes* venoms. Possibly, when rPol d 1 becomes available for clinical laboratory use in parallel with Ves v 1, Ves v 5, and Pol d 5, molecular allergen-based differentiation of dual vespid and *Polistes* venom sensitization may become more discriminating.

Formicidae family: In terms of fire ants, diagnostic *S. invicta* venom has been the most extensively characterized of all the stinging ant venoms. Hoffman et al. [56] identified four allergens in the venom of *Solenopsis invicta* (Sol i 1, 2, 3, 4) (Table 5.1). Sol i 1 comprises 2–5% of the venom, and it shares structural features with vespid venom phospholipase A1 (Ves v 1). Sol i 3 (antigen 5), which is 67% of *S. invicta* venom proteins, cross-reacts with Ves v 5 [56]. Among the *Solenopsis* species, there is extensive documented cross-reactivity of IgE anti-*S. invicta* with phospholipase A1 and antigen 5 venom proteins from *S. richteri* (black imported fire ant), *S. geminata* (tropical fire ant), *S. xyloni* (southern fire ant), and *S. aurea* (desert fire ant) [57, 58]. At present, the fire ant venom allergenic components noted in Table 5.1 are not available for commercial IgE antibody in vivo or in vitro testing.

Discordant Confirmatory Test Results with the Clinical History

The golden rule in allergy diagnostics is that the presence of allergen-specific IgE antibody alone does not definitively prove that the patient has allergic disease without the support of an unequivocal clinical history. When venom-specific IgE is detected by in vivo or in vitro assays using venom extracts or molecular allergens in an individual who has not manifested any allergic symptoms following a sting, the test result has commonly been considered *false positive*. However, it might be better considered as a *clinical irrelevant* but true sensitization since the analytical sensitivity of the skin test and the current IgE antibody autoanalyzers have been optimized based on technical parameters. Negative IgE antibody test results can also occur in a patient with objective allergic symptoms or a definitive history of a severe allergic reaction [10]. In these cases, IgE production may be localized to a target organ and not systemically distributed. Alternatively, low quantities of circulating venom-specific IgE antibody may be present in a serum with a low total IgE that is below the analytical sensitivity of the assay, but where the specific to total IgE ratio may be sufficiently high to sensitize effector cells and elicit mediator release in the presence of allergen [24]. In certain cases, an allergen specificity is in low abundance or missing in the allergen extract. This may prevent circulating IgE antibody from binding, especially if high levels of competing allergen-specific IgG antibody are present. This concern has been addressed by supplementing the extract with the missing or deficient allergen in a recombinant form [18].

Future Perspectives

While both in vivo and in vitro assays have important roles to play in the diagnosis of allergic sensitization, the intradermal skin test remains the diagnostic confirmatory test of choice for evaluating patients with a positive history of an insect sting reaction. In cases where the ID-ST result is discordant with the patient's history or there are confounding issues such as dermatographism or suspected medication interference, in vitro tests for IgE antibody serve as an effective alternative confirmatory test. Singleplex IgE antibody autoanalyzers remain the first choice for venom-specific IgE in vitro testing in routine clinical laboratories due to their worldwide availability and the stability of IgE antibody in serum [18]. Panel testing with multiplex chip-based assays which use venom components will remain an invaluable research tool for epidemiological studies into the foreseeable future. Basophil-based assays as performed in specialized laboratories will serve as important supplemental in vitro assays for clarifying discordant results. Finally, venom extracts will continue to be used as the primary allergen source for both in vivo and in vitro assays. With time, increasing use will be made of recombinant venom allergens to clarify issues of multiple sensitization due to carbohydrate and/or common protein cross-reactivity. Regardless of the confirmatory test performed and venom allergen source used, the ultimate arbiter for the diagnosis of venom-related insect sting allergy is the patient's clinical history [59].

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Chapter 6
Pathologic Findings in Hymenoptera Sting Fatalities

Jessica B. Perkins and Anne B. Yates

Adverse Reactions to Hymenoptera Stings

The body generally responds to Hymenoptera stings in five ways: normal local reactions, large local reactions, systemic anaphylactic reactions, systemic toxic reactions, and unusual reactions [1]. Normal local reactions are a mild non-life-threatening consequence of Hymenoptera stings and the most common occurrence.

Large local reactions involve local swelling >10 cm that lasts longer than 24 h [2]. Large local reactions may be IgE-mediated, cell-mediated, or a combination of both [2]. An adult who experienced a large local reaction due to a sting has about a 10% risk of anaphylaxis in subsequent stings. Children have a much lower risk of developing anaphylaxis after a previous large local sting reaction [3].

Systemic anaphylactic reactions are a potentially life-threatening event. Those patients who have IgE to Hymenoptera venom are at risk for cross-linking of IgE on mast cells and basophils, leading to the release of intracellular substances. Venoms contain multiple protein allergens, and most have enzymatic activity [3]. These molecules can cause pathologic changes involving all organ systems, which can result in death.

Systemic toxic reactions are not IgE-mediated responses. These reactions are caused by massive envenomation from numerous stings, usually involving hundreds or even thousands of stings. The toxic actions of bee venom were shown to trigger an inflammatory response in a mouse model; the pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 were reported as causing systemic inflammation and tissue damage [4].

Unusual reactions can consist of a variety of symptoms that may reflect serum sickness, vasculitis, neuritis, myocarditis, and encephalitis. These reactions are

J.B. Perkins, M.D. • A.B. Yates, M.D. (✉)
Department of Pediatrics, University of Mississippi Medical Center,
2500 North State Street, Jackson, MS 39216-4505, USA
e-mail: jperkins@umc.edu; ayates@umc.edu

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often referred to as unusual because they do not manifest clinically like typical allergic reactions, even though IgE to the venom may be present in the serum [5]. Onset may be days to weeks after the stings and are usually from the tissue deposition of antibody-antigen complexes. Cold-induced urticaria has also been reported, without any initial reaction to the sting [6]. Reisman reports acute disseminated encephalomyelitis starting 8 days after two yellow jacket stings (with headache, and memory and psychomotor dysfunction), acute renal failure after massive stinging of African honeybee (associated with hypotension, hemolysis, rhabdomyolysis, and coagulation abnormalities), myocardial infarction after several wasp stings, diffuse alveolar hemorrhage following a single insect sting, and rhabdomyolysis occurring several hours after a single insect sting [5].

Two Main Categories of Hymenoptera Sting Fatalities

When discussing the pathologic findings in Hymenoptera sting fatalities, it is important to distinguish the two main mechanisms that cause these deaths: Type 1 hypersensitivity reactions and envenomations, also called systemic toxic reactions [7]. A Type 1 hypersensitivity reaction is an IgE-mediated response that occurs when receptor-bound IgE antibodies on the surface of mast cell and basophils cross-link. Due to this cross-linking, substances inside of the mast cell or basophil, such as leukotrienes and histamine, are released and enter systemic circulation. The body can have a pathologic response to these substances, which can subsequently lead to anaphylaxis. Anaphylaxis is a life-threatening allergic reaction that involves two or more organ systems. Several case reports note laryngeal edema, hypotension, and pulmonary congestion as causes of death due to anaphylaxis. Other postmortem lab findings of those victims who succumbed to Hymenoptera sting anaphylaxis were positive for increased levels of serum tryptase [8]. Positive IgE antibody to venom has been reported in the literature, but a report by Hoffman showed that the level of specific IgE antibody to venom is not predictive of the severity of the clinical reaction [9].

The other main cause of Hymenoptera sting fatalities is envenomation. This is caused by a systemic toxicity from large amounts of venom exposure. Those who suffer from envenomation were usually the victims of a massive number of stings at one given time. Envenomation is not IgE-mediated and is not dependent on preexisting allergic conditions [7]. Several case reports describe multiple organ failures from toxic compounds in Hymenoptera venom such as phospholipase A1, hyaluronidase, and antigen 5 [1]. It is noted with fire ant venom there is a unique alkaloid component of the venom that is cytotoxic and can lead to respiratory, cardiac, and neurologic toxic effects in mammals [10]. Pulmonary edema, renal failure, and cardiac failure due to envenomation are well documented in the literature that will be discussed later in this chapter.

Some reports do note that death from organ failure and local swelling due to envenomation could not always be differentiated from anaphylaxis. Because of their

two separate mechanisms of action, presence of tryptase and elevated IgE levels to venom can help with this delineation [8].

Incidence of Insect Sting Allergy Fatalities

It is reported the estimated prevalence of systemic reactions due to Hymenoptera venom allergy in the USA is 0.5–3% [3] and in Europe is 0.3–7.5% [11]. Approximately 0.3–42.8% of these cases were anaphylactic in nature. The mortality due to insect sting anaphylaxis is from 0.03 to 0.48 fatalities per 1,000,000 inhabitants/year [11]. At least 40 fatal sting reactions occur each year in the USA [3, 12] and about 20 fatal reactions from stings in the UK [13]. Half of the fatal reactions that occur have no history of a previous sting reaction [3].

When observing all types of anaphylaxis per year, stinging events caused by Hymenoptera accounted for 1.5–34.1% of cases [14]. It is estimated that around 56–94% of the population is stung by a member of the order Hymenoptera at least once in their lifetime [1, 14]. It is clear that the prevalence of Hymenoptera stings and subsequent life-threatening reactions is not negligible. There is speculation that the incidence is higher than the reported cases shown, as many sting fatalities are not recognized and may be misreported as unexplained deaths [11].

Risk Factors for Fatal Sting Anaphylaxis

Stoevesandt et al. reported 657 consecutive patients referred to a German center for insect venom allergy. Significant risk factors for severe anaphylaxis include elevation of baseline serum tryptase level (greater than or equal to 11.4 ng/mL), time interval from sting to onset of symptoms of less than 5 min, older age, and absence of urticarial lesions or angioedema during anaphylaxis (which occurs with severe hypotension and poor peripheral perfusion) [15].

Other reported risk factors for fatal anaphylaxis to Hymenoptera stings include a history of Hymenoptera allergy, male (men have three times more risk than women), age greater than 40 years, comorbidity with cardiovascular disease, sting site on the head or neck, and bee sting [16, 17]. However, 40% of those who die from Hymenoptera stings have no prior history of previous reactions [16].

Patients with systemic mastocytosis are known to be at increased risk for systemic reactions to Hymenoptera stings. In fact, insect stings are the most common cause of anaphylaxis in patients with systemic mastocytosis [3]. An increased baseline serum tryptase level is associated with a history of more severe sting reactions, and persistently elevated baseline serum tryptase level is a minor criterion for diagnosis of systemic mastocytosis [18]. A normal baseline serum tryptase level does not exclude systemic mastocytosis. Patients with systemic mastocytosis

have a higher chance for severe systemic anaphylaxis without urticaria or angioedema [19].

There may be a greater chance of systemic allergic reaction to an insect sting if multiple stings occur at one time or if there are repeat stings in a short time frame [3].

Pre-existing cardiovascular disease is a significant risk factor for severe and fatal anaphylactic sting reactions [17, 20]. Taking a β-blocker does not seem to increase the risk for a systemic reaction to stings, but may affect the severity of the reaction [11]. Patients taking β-blockers may cause epinephrine resistance, requiring additional doses of epinephrine and intravenous fluids, and glucagon injection to treat anaphylaxis [3]. There are conflicting reports as to whether angiotensin-converting enzyme inhibitors directly increase the risk for systemic allergic reactions to Hymenoptera stings, but as with β-blockers, the main risk may be the underlying cardiovascular disease [3, 11].

Pathologic Findings in Fatal Insect Sting Anaphylaxis

It can be difficult to diagnose anaphylaxis via autopsy because anatomical findings can be nonspecific. Shen et al. report postmortem findings in 28 anaphylactic deaths in Maryland and Shanghai from the years 2004 to 2006 [21]. Laryngeal edema, mucous plugging in the airways, and erythematous skin rashes were observed in some. Pulmonary congestion and edema were present in all cases. Mayer et al. report the serologic findings in postmortem anaphylaxis. This study showed that moderately elevated tryptase was a nonspecific finding in postmortem cases; however, an exceedingly high elevation of tryptase is strongly correlated with anaphylaxis [22].

A report of 56 cases of deaths from anaphylaxis using a registry in the UK showed that 19 of those deaths were from reactions to bee or wasp venom. Of these 19 deaths, 11 were male, and the median age at death was 64 years (age range 28–85 years). Of these 19 sting deaths, 16 were immediate and 3 were delayed deaths. Features suggestive of anaphylaxis were present in only 67% of the cases of immediate sting anaphylaxis deaths. Pathologic findings on postmortem examination included pulmonary congestion or edema in 14 of 19 cases (74%), mucus plugging of bronchi or hyperinflation (37%), upper airway edema (32%), or petechial hemorrhages suggestive of asphyxiation (21%). Only one of these 19 deaths from stings had erythema, urticaria, or edema of the skin (5%) [23]. Lack of specific postmortem findings is thought to be due to sudden, severe cardiovascular collapse [17].

Most fatal cases of insect sting anaphylaxis have an abrupt onset, progressively worsening symptoms, and occur very quickly after the sting(s) [20]. Patients may not have urticaria initially, especially if severely hypotensive. Sometimes urticarial lesions appear during resuscitation, as the blood pressure is restored with increased cardiac output to peripheral tissues, allowing urticaria to appear [17].

Serum tryptase levels in fatal sting anaphylaxis are usually greater than or equal to 44.3 ng/mL, which helps to distinguish death due to anaphylaxis from deaths due to other causes [17]. When postmortem serum is stored frozen at −20 °C, IgE levels and serum tryptase levels are stable and may be measured later. Serum stored at room temperature may still have 50% of its serum tryptase level for up to 4 days [17].

Postmortem serum tryptase levels may be elevated (greater than 11.4 ng/mL) when patients die from other non-anaphylactic causes, including asphyxia, assault, sudden infant death syndrome, sudden cardiac death, trauma, heroin injection, or aortic dissection [17, 24].

Serum platelet-activating factor (PAF) concentration is increased with anaphylaxis, and higher levels are found in fatal anaphylaxis. Platelet-activating factor acetylhydrolase level is decreased in severe anaphylaxis, suggesting there may be insufficient degradation of PAF in severe or fatal anaphylaxis [17].

Features of Death from Hymenoptera Sting Anaphylaxis Versus Other Causes of Anaphylactic Deaths

Most patients who die from insect sting anaphylaxis have a high serum tryptase (>45 ng/mL) [22]. Upper airway edema was more common in deaths from food allergens (62%) than in those from venoms (32%) or drugs (33%). They found that 23 of the 56 anaphylactic deaths had no macroscopic findings on postmortem examination, raising the possibility that many deaths from anaphylaxis may be missed [23].

Clinical features of death from Hymenoptera sting anaphylaxis are similar in some ways to other causes of anaphylaxis. For example, several reports detail respiratory and cardiac compromise as cause of death for anaphylaxis in general. There has been evidence to show that the slower the onset of sting reaction, the less likely the sting is life-threatening. This may not be the case for food anaphylaxis [3]. Food-induced anaphylaxis can occur within a few minutes to a few hours after exposure [25].

As with other causes of anaphylaxis, insect sting-induced anaphylaxis may become more severe with each exposure. However, Golden reports that less than 1% of patients had a reaction more severe than past allergic reactions to insect stings [3].

Case Reports of Reactions to Massive Stings

Kularatne et al. report a 48-year-old man and his 46-year-old wife with deaths 32 h and 9 h, respectively, after a massive attack from hornets. The woman had 40 stings, and the man had 130 sting marks on his skin. Autopsy results showed pleural

effusions, acute pulmonary edema, and red cell casts in their urine. Alveolar tissues were filled with fluid with extravasated eosinophils and neutrophils. Skin biopsy had features of dermal edema. Increased microvascular permeability and acute pulmonary edema were felt to be the primary cause of these two deaths [26].

There have been case reports detailing imported fire ant attacks of the elderly in health-care facilities causing morbidity and mortality. deShazo et al. report a fatality of an elderly individual in a nursing home who suffered more than 500 ant stings [27]. She subsequently developed dyspnea and worsening of her chronic congestive heart failure, which resulted in death 5 days after the incident. deShazo et al. researched case reports of fire ant attacks in patients in health-care facilities and report clinical consequences ranging from no adverse events to anaphylaxis with cardiopulmonary arrest, aspiration pneumonia and subsequent acute respiratory distress syndrome, and death from sepsis [28]. Rupp and deShazo searched the archives of newspapers from 1989 to 2004 in fire ant endemic areas of the USA. Results found 20 stinging victims whose consequences ranged from no serious adverse events to death [29].

Ittyachen et al. report a 32-year-old man presenting with breathing difficulty and decreased urine output. He was stung by multiple wasps the day prior and had developed breathing difficulty immediately after the incident. He was treated at a local hospital and discharged home in seemingly good condition. The next day he developed increased work of breathing and oliguria. He had an extensive intensive care unit stay for multi-organ dysfunction including severe renal failure, hemolysis, rhabdomyolysis, and liver dysfunction. He was successfully discharged after 3 weeks of fluid, steroid, antihistamine, and hemodialysis therapy [1].

Yamazaki et al. report a 66-year-old man that was stung over 30 times by wasps. He was observed in the emergency room and initially showed no signs of anaphylaxis. Twenty hours after the stinging event, he developed vomiting, abdominal pain, rhabdomyolysis, acute renal failure, and nonocclusive mesenteric ischemia. He was thought to have delayed toxin-mediated systemic reaction from the massive wasp envenomation [30].

Nandi and Sarkar report a 9-year-old boy who developed anuria 2 days following an attack from a swarm of wasps. As previously mentioned, the venom from Hymenoptera stings can have hemolytic, neurotoxic, and vasoactive properties. These characteristics can lead to intravascular hemolysis and rhabdomyolysis. However, in this case it was the venom itself that caused a hypersensitivity-related acute interstitial nephritis leading to acute renal failure. Hemolysis and rhabdomyolysis were reasonably ruled out in this case [31].

Summary

The pathologic findings of Hymenoptera sting fatalities have a wide range of variability. Organ failure such as respiratory congestion, heart failure, renal failure, and vascular permeability and hypotension seem to be the most commonly reported

causes of death. Because anaphylaxis is a clinical diagnosis, it cannot always be diagnosed through autopsy findings. Multi-organ failure that results from envenomation is likely due to the toxic systemic effects rather than IgE-mediated anaphylaxis. Postmortem labs such as tryptase levels and IgE to Hymenoptera venom can help discern which cases were allergic in nature, but this is not an absolute finding.

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Chapter 7
Treatment of Hymenoptera Reactions: Acute and Prophylactic

David F. Graft

Treatment of Acute Sting Reactions

Minor local swelling and pruritus are expected following an insect sting and can be treated with ice and antihistamines. If present, the imbedded stinger should be flicked off with a scraping motion. Schumacher [1] showed that essentially the entire honeybee venom load is injected in less than 20 s; one should quickly remove the stinger.

If a generalized reaction occurs, epinephrine is the keystone of management. Epinephrine halts the further release of mediators and reverses many of the effects of already released mediators. An intramuscular injection of epinephrine usually produces prompt resolution of symptoms. The dose of epinephrine is 0.3–0.5 mg for adults and 0.01 mg/kg for children. The regular adult versions of the epinephrine auto-injector contain 0.3 mg. The Jr versions contain 0.15 mg. The dose may be repeated in 10–15 min if necessary. An oral or parenteral antihistamine such as diphenhydramine is also usually given. It may lessen urticaria, but in more severe or progressive reactions, its use should not delay the administration of epinephrine.

Epinephrine may be ineffective in profound anaphylactic shock unless the functional hypovolemia is corrected with intravenous fluids [2]. Severe reactions often require treatment with oxygen, H_2 antihistamines, and pressor agents. Corticosteroids are widely used but they have delayed onset of action. Intubation or tracheostomy is indicated for severe upper airway edema not responding to therapy. Close observation is essential. Overnight hospitalization is suggested for patients who have experienced severe reactions or who have complicated medical problems.

D.F. Graft, M.D.
Department of Asthma and Allergic Diseases, Park Nicollet Clinic, Minneapolis, MN, USA
e-mail: graftd@parknicollet.com

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Decreasing Future Reactions

Future stings can be avoided by taking common sense precautions to significantly reduce exposure. Shoes should always be worn outside. Someone else should eradicate nearby hives and nests. Patients should avoid gatherings where outdoor food and drink (especially canned soda) are found, garbage cans, and garden work. Insect repellents have little or no effect, and avoidance of brightly colored clothing has been shown not to be of clear benefit [3].

Emergency Epinephrine

To encourage prompt treatment, epinephrine is available in emergency kits for self-administration. These are used by insect sting-allergic individuals immediately after the sting to "buy time" to get to a medical facility. The epinephrine auto-injector (0.3 mg & 0.15 mg epinephrine) offer a concealed needle and a pressure sensitive spring-loaded injection device that make them suited for patients and families who are uncomfortable with the injection process. Usually, the regular adult versions of these devices are prescribed for children greater that 50–55 lb. Many patients in urgent care settings do not receive a prescription for self-administered epinephrine or referrals to allergists for consideration of VIT [4]. Patients who are receiving maintenance injections of VIT are advised that emergency self-treatment will probably not be required; however, they should have the devices available if they are distant from medical facilities. A medic alert or other medical information bracelet is also advised.

How to Initiate Venom Immunotherapy

The study of sensitivity to insect stings holds a unique position in the field of allergy, and because of the usually specific and well-recalled times of exposure, it serves as a model for the development, natural history, and treatment of allergic disorders. In 1925, Braun [5] described a typical patient with insect sting sensitivity and his use of insect venom for diagnosis and treatment. Although this initial therapy used the posterior one-eighth inch of the insect to increase the yield of venom, that stipulation was later ignored, and for decades, immunotherapy with whole-body extract was used for the treatment of patients with reactions to insect stings [6]. Loveless [7] reported the use of extracts of venom sacs, and others showed that whole-body insect extracts lacked diagnostic specificity [8, 9]. In 1978, Hunt and coworkers from Johns Hopkins reported a challenge sting trial showing the vast superiority of venom immunotherapy (VIT) when compared with whole-body and placebo injections [10]. Immunotherapy with venom is the most effective treatment in the field of allergy. Up to 98% of

venom-treated patients have no reaction when stung. The remaining patients are more appropriately considered partial successes rather than treatment failures since they usually have reactions that are less severe than those they have had before.

Insect venoms for immunotherapy have been available commercially since 1979. Venom injections greatly reduce the likelihood of serious allergic reactions. Patients on VIT experience an improvement in quality of life by reducing anxiety and allowing them to participate in the outdoor activities that they prefer. Guidelines and reviews for the management of insect venom sensitivity have been published [11–14]. Venom injections have proved to be very safe with a low occurrence of injection-induced systemic reactions. Unfortunately, even after nearly 40 years of use, many patients who should initiate VIT are not referred to allergists for evaluation despite data that demonstrate the lack of effectiveness of single or multiple doses of epinephrine [2].

Patient Selection

Hymenoptera stings can cause systemic allergic reactions which may be mild with only cutaneous symptoms (pruritus, urticaria, angioedema of the eyes, lips, hands, etc.) or severe (anaphylaxis, life-threatening) with laryngeal edema, bronchospasm, and hypotension manifested by an uncomfortable feeling in the throat, gagging, difficulty swallowing, voice change, inspiratory stridor, chest tightness, wheezing, cough, dizziness, tunnel vision, or syncope. Adults often describe a metallic taste in their mouth or an aura of impending doom, as if they are going to die. Although urticaria/angioedema is generally considered characteristic of an allergic reaction, the absence of urticaria or angioedema during anaphylaxis may be a risk factor for severe anaphylaxis [15].

VIT is a safe, highly effective method of preventing future systemic sting reactions in insect-allergic patients. An untreated adult's risk of a recurrent systemic reaction is about 30–60%. After VIT is initiated and maintenance dose achieved, the risk is reduced to as low as 2%. The effectiveness of VIT in patients with past systemic reactions was demonstrated in several observational studies and a small number of randomized, controlled trials. A systematic review included seven trials, but the reviewers combined patient groups that did not have equivalent types of reactions or natural histories [16]. The Hunt et al. study [10] involved 60 adults with systemic reactions of similar severity who were treated with either VIT using a maintenance dose of 100 μg of venom, immunotherapy with Hymenoptera whole-body extract, or placebo injections for 6 weeks. In-hospital challenge stings resulted in systemic reactions in 1 of 18 (6%) in the VIT group, compared with 7 of 11 (64%) in the whole-body extract group and 7 of 12 (58%) in the placebo group. Protection from recurrent systemic allergic reaction appears to be established in outpatients within a week of reaching maintenance dose [17].

The selection of patients for VIT is determined by the likelihood that a future sting will cause an allergic reaction, which is based on the clinical history and the results of venom skin tests (VSTs) and occasionally venom-specific IgE. The risk of recurrence

is higher for adults than for children, higher for those allergic to honeybee rather than vespid venom, and higher for patients whose previous reactions were more severe [18–20]. A careful history discloses the type, degree, and time course of symptoms and often identifies the culprit insect. An individual who has experienced a sting-induced systemic reaction should be referred to an allergist who will perform skin tests with dilute solutions of honeybee, yellow jacket, yellow hornet, white-faced hornet, and *Polistes* wasp venoms and, if indicated, imported fire ant whole-body extract [21, 22]. Measurement of venom-specific IgE cannot replace VST but may provide additional information [23]. To date, fire ant venom has only been available in small research quantities; fortunately, the fire ant whole-body extract material contains a significant amount of venom and has been successfully used for skin testing and treatment.

Table 7.1 lists the indications for VIT. At particularly low risk are children who have had reactions limited to the skin in which there is only a 9% rate of subsequent systemic reactions involving the skin [18]. This study presented 242 children (aged 2–17 years) who had experienced generalized cutaneous reactions to insect stings. All had positive skin tests to venom. VIT was administered to 68 children. About one-half of children were randomized to the treatment they received, while parent preference determined the treatment of the others. During 4 years of observation, there were 280 accidental stings in 122 of the children. In the children receiving VIT, 1.2% of the stings resulted in systemic symptoms, compared with 9.2% in the untreated group, but none of these were more severe than the original event, and 16 of 18 reactions were milder. Thus, although VIT is effective in children with generalized cutaneous reactions, it does not appear to be necessary. In contrast, children who have had moderate or severe reactions should start VIT; those who do not start VIT have a significantly higher risk of reaction as adults, estimated to be 30% [24].

VIT is recommended for an adult who has experienced a moderate to severe sting-induced systemic reaction. Most individuals have a stereotypic response to a sting with the symptoms on subsequent stings closely resembling the first episode [25]. In the most recent American guideline [11], VIT is not generally

Table 7.1 Selection of patients for immunotherapy

Reaction to sting	Result of skin test or RAST	Venom immunotherapy?
Systemic, non-life-threatening, immediate, generalized urticaria, angioedema, erythema, pruritus	+ or −	No[a, b]
Systemic, life-threatening, possible cutaneous symptoms but also respiratory symptoms (laryngeal edema or bronchospasm) or cardiovascular symptoms (hypotension, shock)	+	Yes
Systemic reaction	−	No[c]
Large local (>4 in diameter, >24-h duration)	+ or −	No[a]
Normal (<2 in, <24-h duration)	+ or −	No

[a]General recommendations

[b]It is unknown whether this recommendation applies to imported fire ant hypersensitivity

[c]Patients with a history of a systemic reaction but negative tests for venom-specific IgE may be at risk for a future systemic reaction for unknown reasons. See further discussion in text

encouraged in the United States for patients older than 16 years of age with systemic reactions limited to the skin, similar to guidelines from Europe that have recommended not treating this group [26, 27]. Contravening risk factors (concomitant cardiovascular disease or certain medications such as ACE inhibitors or beta-blockers, elevated baseline tryptase, a high likelihood of future stings (occupational or hobby exposure), or effect on quality of life) need to be considered. When only a mild reaction has occurred, the physician and patient may jointly decide whether to embark on a course of VIT. There are no studies comparing VIT vs no VIT in these patients to guide this decision. There are questions regarding whether younger adults and older adults should have the same recommendations, especially since deaths from insect stings increase with age (for 1980–1999, US data: 20–29yo, 41 deaths; 30–39yo, 112 deaths; 40–49yo, 196 deaths; 50–59yo, 217 deaths; 60–69yo, 207 deaths; greater than 70yo, 131 deaths) [28]. Individuals who have had severe allergic reactions should be advised not to depend only on avoidance and availability of epinephrine but also to receive venom injections. Patients with a history of systemic reactions have a reduced quality of life [29]; this is not improved by having self-administered epinephrine but is with VIT [30].

A large local reaction or extreme swelling extending from the sting site, usually peaking at 48–72 h after a sting and lasting 1 week or more, is usually the result of an IgE-mediated late-phase reaction. Individuals with large local reactions are generally not placed on VIT. The risk of anaphylaxis to future stings is about 5–10% in patients who have had large local reactions to insect stings; many, however, develop large local reactions again [31–33]. Because the risk of a systemic reaction is relatively low in patients who have previously had large local reactions, diagnostic testing and VIT are generally not required in such patients. Large local reactors might be considered for VIT (and therefore testing) for quality-of-life reasons and to reduce the morbidity of frequent or unavoidable reactions [34].

Many patients with large local reactions have evidence of a high level of venom sensitivity. Because it is also true that some patients with severe anaphylaxis have low levels of venom sensitivity, the clinician should remember that the level of skin test reactivity (and venom-specific IgE) is poorly correlated with the risk of systemic reactions [21]. A positive VST or evidence of venom-specific IgE in the absence of a sting-induced systemic reaction is not an indication for therapy. Approximately 25% of the general population may have such evidence of sensitization to venom antigens, apparently resulting from past stings. This is usually transient, disappearing in 1–3 years [35]. Patients with negative skin tests are not candidates for venom imunotherapy (see Venom Skin Test-Negative Patient section below).

Other factors may influence the decision concerning the need for VIT. Those with an increased risk of being stung, such as landscapers or other individuals who engage in outdoor activities, especially those that take them far away from available medical care, dispose the physician toward initiating venom treatment. Part of the morbidity associated with stinging insect allergy includes psychological effects of anxiety in the victim and family because of the threat of a sudden “unpreventable” systemic reaction. This high level of anxiety has frequently been exacerbated by a physician warning that “the next sting may be your last.” Actually, most victims exhibit an individual pattern of anaphylaxis that varies only slightly in severity from

one sting to another. Some investigators have proposed that a diagnostic sting challenge be used to select patients for VIT [25]. Patients who did not react would not be placed on VIT. Other reports, however, have described individuals who tolerated one sting challenge, but reacted later to a second challenge [36, 37]. In the United States, the use of sting challenges to determine VIT candidates is impractical, and most physicians believe that a challenge sting presents too great a risk [38, 39], especially for patients with life-threatening reactions (i.e., hypotension). Indeed, in one study, a life-threatening reaction recurred in 15% of subjects with histories of severe reactions [25].

Baseline tryptase measurements are helpful in assessing a patient's risk for systemic reactions in response to the dose escalation phase of VIT. Baseline levels should be measured after the sting reaction has fully resolved and the patient is in their usual state of health. Baseline serum levels above 11.4 ng/mL should prompt an evaluation for a mast cell disorder. In a prospective study of patients receiving honeybee or vespid VIT, serum tryptase correlated with the risk of adverse systemic reactions to therapy. An increase in baseline tryptase from 4.2 to 20 ng/mL heightened the risk for a systemic reaction by a factor of approximately 3.8 [40].

The measurement of baseline tryptase is also useful in assessing other aspects of a patient's risk. Specifically, elevated baseline tryptase is associated with more severe reactions to stings in general, more frequent treatment failure (i.e., failure to achieve protection), and a greater risk of relapse after stopping VIT [40–42].

The Venom Skin Test-Negative Patient (Never Say Never)

The patient with a history of a sting-induced systemic reaction and negative VSTs presents with a unique challenge. It had been commonly assumed that negative VST responses indicated that there is no risk of systemic reaction to a sting. Golden et al. [43] reported, however, that of 307 subjects with a history of a sting-induced systemic reaction who underwent VST 99 (32%) had negative VST. Of these, negative RAST was present in 56 patients; 36 had low (1–3 ng/mL) RAST; and 7 had high positive RAST (4–243 ng/mL). Sting challenges in 14 of 56 patients with negative VST and negative RAST resulted in two (14%) systemic reactions. Sting challenges in 37 of 43 patients with negative VST and positive RAST caused nine (24%) systemic reactions. Combined, the negative VST group had a systemic reaction rate of 22%, which was similar to the rate of 21% found in those with positive VST studied at the same time. In summary, a patient with a convincing history of a sting-induced systemic reaction should have VST. If these are negative and the reaction was severe, venom-specific IgE should be measured and VIT initiated if positive. If negative RASTs are obtained, the VST should be repeated 3–6 months later [44, 45]. Regardless of the VST and venom-specific IgE determinations, the patient should practice usual precautions of insect sting avoidance and should carry antihistamines and injectable epinephrine.

Venom Selection

The venoms used for immunotherapy are the same as those used for skin testing (honeybee, yellow jacket, yellow hornet, white-faced hornet, wasp, and fire ant whole-body extract if applicable). The regimen begins with an injection of 0.01 μg and advances weekly to 100 μg (0.5 of 1:100 wt/vol for fire ant whole-body extract). The maintenance dose of 100 μg is given every 4 weeks for a year, after which the interval is lengthened to 6 weeks for a year and then 8 weeks in most patients.

The choice of venoms is based primarily on VST results, and to a lesser extent on clinical history and patterns of venom cross-reactivity. It is currently recommended that immunotherapy include all venoms giving a positive skin test, the aim being to give the maximum security to the patient [46]. Even a patient who has reacted to only one type of insect should not be left with lingering doubts about future stings by other insects to which he or she shows skin test sensitivity. The most common culprit in North America is the yellow jacket, except for some areas of the south central and southwestern states, where wasps, honeybees, and fire ants are predominant. In some cases, the skin tests are positive to only one or two venoms. Most yellow jacket-allergic patients also have positive skin tests to yellow hornet and white-faced hornet venoms, however, and consequently require treatment with mixed vespid venom containing the full dose of each venom (yellow jacket, yellow hornet, and white-faced hornet). Half of these patients are also positive to *Polistes* wasp venom and receive this in addition. RAST inhibition studies can disclose whether patients are sensitive to a cross-reacting allergen or multiple unique allergens [47]. The allergenic cross-reactivity of the four vespid venoms has been demonstrated, as has the fact that most patients with multiple vespid venom sensitivity can be fully protected by immunotherapy with yellow jacket venom alone [48]. The use of recombinant venom allergens to resolve honeybee and yellow jacket double positivity has been reported [49].

The maintenance dose for venom immunotherapy is 100 μg per venom. This dose was initially chosen because it represented approximately twice the venom content of a honeybee sting. One must remember that in many studies, yellow jacket-allergic patients were treated with mixed vespid venom because of concomitant sensitivity to yellow and white-faced hornet. Treatment with this 300 μg total vespid dose conferred complete protection in 98% of patients, compared with 90–95% in patients treated with single vespid venoms (100 μg dose) and 75–85% for honeybee venom [19, 46, 50]. Patients who are not adequately protected by the 100 μg per venom dose are often protected with higher doses [50]. Conversely, some studies have shown that a lower dose can be effective. One investigator has reported his 10-year experience with a maintenance venom dose of 50 μg [48]. The question of whether or not a lesser dose would suffice was examined in a group of patients who received a 50 μg maintenance dose. It was found that with the reduced venom dose, only 79% of adult subjects were protected from challenge sting-induced systemic reactions [51]. A 50 μg maintenance dose for honeybee VIT has

been reported effective in children [52]. However, nearly all patients are treated with a 100 μg per venom dose.

Bernstein and colleagues [53] described a 2- to 5-h regimen of rush VIT in which 10 gradually increasing doses were administered every 10–15 min to achieve a total dose on day 1 of about 55 μg per venom followed by doses of 70, 80, 90, and 100 μg on days 3, 7, 14, and 21, respectively. Only 4 of 33 patients had reactions on day 1, and all were mild. These patients subsequently tolerated natural stings without incident. Ultra-rush VIT (accomplished in 4–8 h) is associated with higher frequency of systemic reactions [54].

There is uncertainty about the safety of ACE inhibitors and beta-blockers during VIT [55]. Patients receiving VIT and concomitant treatment with ACE inhibitors appear to be at risk for more severe anaphylaxis in response to treatment [55–57]. However, there does not appear to be a correlation between ACE inhibitor therapy and a higher overall incidence of anaphylaxis [56, 57]. In patients taking adrenergic blockers, data are limited [58, 59]. Other forms of allergen immunotherapy, such as that for respiratory allergies, are not usually administered to patients in whom beta-blocker therapy is essential (e.g., individuals with significant cardiovascular disease). However, recommendations for VIT in such patients are different because of the life-threatening nature of untreated Hymenoptera anaphylaxis, especially in patients with cardiovascular disease. In summary, patients on VIT should discontinue beta-blockers if possible, but if not, then VIT should proceed with caution [11].

How Does Venom Immunotherapy Work?

The mechanism of action of immunotherapy is only partially understood. The development of allergic sensitization to venom requires the sting-induced production of venom-specific IgE antibodies that are bound to tissue mast cells and circulating basophils. A subsequent sting may then result in the binding of venom antigens to the IgE molecules followed by the release of mast cell and basophil mediators of anaphylaxis (histamine, leukotrienes, and others). It should be remembered that venom vaccines used to immunize patients with insect sting hypersensitivity are not clinically or immunologically identical to the venom injected by live stings. In the initial controlled trial of VIT, some patients tolerated injections of 100 μg of venom administered subcutaneously immediately before challenge stings that caused severe anaphylaxis to challenge stings [2, 10].

Successful VIT is associated with many humoral and cellular immunologic changes [60–64]. VIT results in significant changes in venom-specific IgE and IgG antibody levels. IgE rises first, peaking at 8–12 weeks, before declining slowly over 3–5 years to pretreatment levels. The IgG level usually increases, peaking at 2–4 months, and then is fairly constant over 5–6 years of treatment in children. Children have a more vigorous IgG response, but analysis of venom-specific IgG levels and the child's age, body weight, or surface area failed to show any correlation [65].

The production of antigen-specific IgG (blocking) antibodies has been considered the possible means of immunotherapeutic improvement. Lessof and coworkers [66] demonstrated that honeybee-allergic patients could tolerate challenge stings after passive immunization with the gamma globulin fraction of pooled beekeeper's serum that contained a high blocking antibody titer. The serum level of venom-specific IgG has been inversely correlated with the likelihood of challenge sting-induced systemic reactions in patients on VIT [67]. After 4 years of VIT, however, that correlation no longer held true. Other immunologic changes occur that could also influence the development of protection to insect stings. VIT seems to influence the T-cell phenotype away from the Th2 type, which produces interleukin-4 and interleukin-5, and toward the Th1 type, which produces interferon-γ [68, 69], or a regulatory type of T response with expression of interleukin-10 and the production of IgG4 [70].

Management of Venom Immunotherapy

Safety of Venom Immunotherapy

VIT generally is well tolerated. Most patients receive their injections in an allergist's office until the maintenance dose is reached. Patients should remain for 30 min after each injection in a setting equipped to handle a systemic reaction. About 3–12% of patients have treatment-induced systemic reactions that generally are mild and occur in the early phases of VIT [71, 72]. The reaction rate is no higher than seen in conventional pollen immunotherapy when effective immunizing doses are used [73]. If a systemic reaction occurs, the regimen is interrupted. One-half of the dose that resulted in a systemic reaction is given the next week, and, if tolerated, the schedule is resumed. Pretreatment with antihistamines reduces VIT reactions and may improve the efficacy of VIT [74, 75]. A less serious, but more frequent, problem with VIT is the large local reactions that occur in 25% of children and 50% of adults, usually at doses about 20–30 μg. Although bothersome, they do not predict an increased risk of future systemic reactions to treatment, and usually the best way to avoid them is to reach higher doses by proceeding with the injections regimen.

No long-term toxicity or side effects have been associated with VIT thus far. Graft and coworkers [14] noted that 3–6 years of VIT in children was not associated with abnormalities in histories, physical examinations, or laboratory analyses (hematologic and chemical surveys and urinalyses) [76]. Few reports of VIT use during pregnancy have been published. Schwartz and coworkers [77] discussed 22 pregnancies in 15 women that resulted in 19 normal children, 1 first-trimester miscarriage, 1 miscarriage secondary to placenta previa, and 1 child with multiple congenital abnormalities of unknown cause. This rate of less than optimal outcome for pregnancy was not higher than expected with pregnancy in normal population. One report noted that VIT during pregnancy did not result in allergic sensitization to venom in the child [78].

Interval Between Venom Injections

The maintenance dose of 100 μg is given every 4 weeks for a year; the interval is usually lengthened to 6 weeks during the second year and to 8 weeks during the third year of treatment [11]. Investigators from Israel described 160 individuals who had the maintenance interval lengthened to 3 months [79]. A subgroup of 47 reached the 3-month maintenance interval only 4.5 months after the maintenance dose was reached. Ninety-three stings in 80 patients on 3-month maintenance interval VIT resulted in four skin reactions (one occurred in one of the few patients on a 50-μg maintenance dose). After VIT was stopped in these patients, 65 stings in 46 patients resulted in four reactions (6.2% per sting, 8.7% per patient). Extending the interval between injections reduces the cost of VIT [80, 81]. These authors reported that a 6-month maintenance interval for venom immunotherapy was not adequate to maintain protection [82].

Monitoring of Venom Immunotherapy

Most patients begin therapy with IgG levels less than 1 μg/mL (usually undetectable), but occasionally they may be elevated because of the previous sting. IgG levels induced by the first 4–6 months of therapy are usually between 5 and 20 μg/mL, with higher levels observed in children and when multiple venoms are administered [67]. Among immunized patients, those who continue to react to stings generally have exceptionally small increases in their venom-specific IgG antibodies. Because there are so few treatment failures with VIT, it may not be cost-effective to perform venom IgG antibody assays on all immunized patients [83].

At follow-up visits, usually annually, the patient's VIT schedule should be reviewed, noting dose and frequency of injections, local or systemic reactions to injections, and any stings (and their outcome) that may have occurred since the last visit. VSTs may be repeated every several years. Over time, VSTs tend to decline and become negative in a significant proportion of patients. In children, Graft and coworkers [84] found that 45% of those who had received 3–6 years of VIT developed negative VSTs to one or more venoms, whereas in adults, Golden and coworkers [85] reported that 20% had negative VSTs after 5 years and 50–60% after 7–10 years.

Honeybee Allergy Versus Vespid Allergy

Honeybee sensitivity is generally a more difficult problem than vespid venom sensitivity. Researchers in the Netherlands stung 324 patients and found that patients with history of honeybee sting reactions were twice as likely as those with yellow

jacket sensitivity to react to challenge stings (52% versus 25%) [25]. Once VIT is commenced, Müller reported that honeybee-sensitive patients have more reactions to VIT (41% versus 25%) than those on vespid VIT [19]. Of concern, honeybee VIT is less effective in preventing future sting-induced systemic reactions (77% versus 91%) [19]. Finally, even after VIT is stopped, patients who received honeybee VIT are more than twice as likely to react to challenge stings (17% versus 4–8%) delivered 1–2 years after VIT discontinuation [86].

Conclusion

Venom immunotherapy reduces the risk of a recurrent systemic reaction in insect-allergic patients from 30–60% to as low as 2%. It has been the most successful type of allergen immunotherapy employed by allergists. Unfortunately, venom immunotherapy remains underutilized primarily because of the lack of awareness of this modality of treatment by many emergency and primary care providers. There remain questions regarding more accurate diagnosis of which patients are at risk for future reactions, particularly identifying patients at risk for their first serious reaction, and progress continues on the subject of starting and stopping venom immunotherapy.

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Chapter 8
Adverse Reactions to Skin Testing and Immunotherapy with Hymenoptera Venoms and Whole-Body Extracts

Robbie Pesek and Richard F. Lockey

Introduction

A diagnosis of Hymenoptera allergy is based on a history of an allergic reaction following an insect sting. It should be confirmed with appropriate in vitro or in vivo testing. Venom immunotherapy (VIT) is recommended for IgE-mediated Hymenoptera allergy as is whole-body extract (WBE) for imported fire ant (IFA) allergy. Both forms of therapy protect against developing future sting-induced systemic allergic reactions (SARs). Skin testing and VIT can result in adverse reactions; therefore, potential factors that increase the risk for the subjects treated must be considered.

Adverse Reactions Associated with Skin Testing

Skin testing by the skin prick test (SPT) or intradermal (ID) methods can be associated with mild discomfort and generate a local wheal with erythema and pruritus that may be uncomfortable for the test subject. These reactions typically develop within minutes of testing and resolve over the following hour or two. Delayed or late phase reactions at the site of the skin test may develop after several hours or even days but commonly resolve without sequelae. The incidence of reactions

R. Pesek, M.D. (✉)
University of Arkansas for Medical Sciences and Arkansas Children's Hospital,
13 Children's Way, Slot 512-13, Little Rock, AR 72202, USA
e-mail: rdpesek@uams.edu

R.F. Lockey, M.D.
Division of Allergy and Immunology, Department of Internal Medicine, University of South Florida Morsani College of Medicine, Tampa, FL, USA

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associated with SPT is low. In the National Health and Nutrition Examination Survey (NHANES), 16,204 subjects ages 6–74 years underwent routine medical procedures including body measurements (height, weight, body circumference, and skinfold size), venipuncture, and SPT (pet danders, dust mites, *Alternaria*, Bermuda and rye grasses, oak, and giant/short ragweed). The rate of any adverse reaction, including allergic and nonallergic reactions, was assessed and occurred with 0.04% of SPT (no SARs) compared to 0.49% with venipuncture [1].

SARs during SPT and ID testing also are rare. Valyasevi et al. reviewed over 16,000 SPT and 1806 ID tests in 18,311 subjects; six SARs occurred with a rate of 33 per 100,000 skin tests performed, all of which occurred in subjects with asthma. Rates of SARs for SPT alone and SPT/ID together were 30 and 55 per 100,000 tests performed, respectively [2]. These results were confirmed in three other studies. Bagg et al. found that SARs occurred in 3.6% of subjects who underwent SPT and ID testing. The rate of SARs associated with SPT was 0.4% and ID testing was 3.2% [3]. In a retrospective study by Swender et al. of skin testing during a 13-year period, over 28,000 SPT and 18,000 ID tests were performed. Although the overall rate of SARs associated with testing was not described, only six subjects (0.02%) had SARs severe enough to require treatment with epinephrine (rate of administration, 20 per 100,000 SPT visits) [4]. In a 6-year prospective analysis of adverse reactions associated with SPT, SARs occurred at a rate of 77 per 100,000 subjects tested with females and subjects with asthma more commonly affected [5]. There is a low rate of SARs with skin testing in children. Norrman et al. performed SPT in approximately 6000 children and found the risk of a SAR to be 0.12%. Factors that increased the risk of a SAR included age less than 1 year (relative risk (RR), 6.28) and active atopic dermatitis (RR, 16.98) [6]. Although the overall risk of SARs with skin testing is low, fatalities have been reported. In a review of 30 fatalities associated with skin testing and allergen immunotherapy (AIT), six were associated with skin testing, five of which occurred when ID testing was performed without prior SPT [7].

The rate of SARs associated with testing to Hymenoptera venoms is also low. In a study of 3236 subjects with either a local or SAR to a Hymenoptera insect, only 2% experienced a systemic reaction during skin testing. Twenty percent of these reactions were considered vasovagal, and 9% were not consistent with an IgE-mediated reaction; thus the rate of a SAR with venom SPT and ID testing was estimated to be 1.4%. Of these, 0.25% were considered severe, involving hypotension, shock, or loss of consciousness [8]. Testing to IFA utilizing WBE also appears to be relatively safe. Thirty-three IFA-allergic subjects and a similar number of age-matched controls underwent testing with IFA WBE. No adverse reactions were seen following SPT, although ID testing at higher WBE concentrations was associated with development of delayed-onset large local reactions (LLRs) in 53% of controls [9].

These studies confirm that the risk of SAR following SPT and ID testing to allergens, including Hymenoptera venoms and IFA WBE, is low. Certain risk factors, including young age, female gender, and uncontrolled atopic diseases, may increase the risk during such testing; thus, the risk/benefit ratio should always be considered before such testing is performed. ID testing should not be performed without prior SPT because of the increased risk of potential SARs or a fatal reaction.

Adverse Reactions Associated with Venom Immunotherapy and Imported Fire ant Whole-Body Extracts

Characterizing Adverse Reactions

A variety of adverse reactions can occur during VIT. Local reactions at the site of injection can consist of erythema, edema, pruritus, and mild discomfort. Although common, these reactions typically self-resolve and do not require treatment. LLRs also occur and involve skin swelling to a larger area, usually more than 10 cm in size. These reactions may take longer than 24 h to resolve but do not require VIT dose adjustments or cessation of treatment as they do not appear to be a risk factor for future SARs associated with VIT or IFA WBE.

SARs occur with VIT and IFA WBE and may be associated with significant morbidity and even mortality. In 2008, a joint effort between the American Academy of Allergy, Asthma, and Immunology (AAAAI) and the American College of Allergy, Asthma, and Immunology (ACAAI) in conjunction with the World Allergy Organization (WAO) and Immunotherapy Surveillance Safety Group of the European Academy of Allergy and Clinical Immunology (EAACI) led to the development of a standardized grading system for SARs that occur during AIT [10] (Fig. 8.1). This grading system not only provides consensus recommendations for grading SARs but also treatment of these reactions to reduce potential morbidity and mortality.

Systemic Allergic Reactions

Multiple studies have addressed the frequency of SARs during VIT. The first major report summarized data from 3236 subjects with Hymenoptera allergic reactions of which 1410 underwent treatment with VIT over 3 years. One-hundred and twenty-seven subjects experienced a total of 327 SARs (1.9 SAR per subject) of which 59% were considered mild, 32% moderate, and 9% severe. The severity of a SAR did not correlate with the severity of the incident sting, degree of sensitivity on skin testing, or the total dose of venom given. Subjects were more likely to experience a SAR during the buildup phase and if they were being treated with honeybee (HB) or wasp venom. Sixty-four percent of reactions occurred prior to achieving maintenance dosing with the highest risk of SAR seen between 1 and 50 μg of venom [11]. Youlten et al. followed 109 subjects prospectively to determine the frequency of SARs during treatment with HB and wasp VIT. SARs occurred with 7.5% of injections during the buildup phase and decreased to 2.1% once maintenance was reached. Of these reactions, 0.47% were considered severe enough to require epinephrine. Over 80% of reactions occurred within 30 minutes of dosing, while 90% occurred within 1 h [12]. Lockey et al. confirmed that most reactions occur within 30 min of dosing in their review of deaths associated with skin testing and AIT [7].

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Symptom(s)/sign(s) of 1 organ system present **Cutaneous** - Generalized pruritus, urticaria, flushing, or sensation of heat or warmth **or** Angioedema (not larygeal, tongue, or uvular) **or** **Upper respiratory** - Rhinitis (eg, sneezing, rhinorrhea, nasal pruritus and/or nasal congestion) **or** - Throat clearing (itchy throat) **or** - Cough perceived to originate in the upper airway, not the lung, larynx, or trachea **or** **Conjunctival** - Erythema, pruritus, or tearing **or** **Other** - Nausea, metallic taste, or headache	Symptom(s)/ sign(s) of more than one organ system present **or** **Lower respiratory** - Asthma: cough, wheezing, shortness of breath(e.g., ↓FEV_1 or PEF by < 40%) responding to an inhaled bronchodilator **or** **Gastrointestinal** - Abdominal cramps, vomiting, or diarrhea **or** **Other** - Uterine cramps	**Lower respiratory** - Asthma (e.g., ↓FEV_1 or PEF by 40%) NOT responding to inhaled bronchodilator **or** **Upper respiratory** - Laryngeal, uvula, or tongue edema with/without stridor	**Lower or upper respiratory** - Respiratory failure with/without loss of consciousness **or** **Cardiovascular** - Hypotension with/without loss of consciousness	Death

Fig. 8.1 WAO SAR grading table [10]. Patients may also have a feeling of impending doom, especially in grades 2, 3, and 4. Note: Children with anaphylaxis seldom convey a sense of impending doom, and their behavior changes may be a sign of anaphylaxis, e.g., becoming very quiet or irritable and cranky. Scoring includes a suffix that denotes if and when epinephrine is or is not administered in relationship to onset of symptom(s)/sign(s) of the SR: a, ≤ 5 min; b, >5 min to 10 min; c, >10 to ≤20 min; d, >20 min; z, epinephrine not administered. The final grade of the reaction will not be determined until the event is over, regardless of the medication administered. The final report should include the first symptom(s)/sign(s) and the time of onset after the subcutaneous allergen immunotherapy injection*** and a suffix reflecting if and when epinephrine was or was not administered, e.g., grade 2a; rhinitis: 10 min

Females receiving HB venom were more likely to have a SAR compared to those receiving wasp venom (60% versus 21%), and age and atopic status did not increase this risk. Stoevesandt et al. estimated the risk of a SAR to be lower in a retrospective review of 818 buildup cycles of VIT with over 85,000 doses administered. SARs occurred in 3.4% of cases with the majority of these reactions limited to the skin. Only 0.6% were considered moderate to severe, usually associated with HB venom [13]. SARs occurred in 20% of subjects, 1.9% during the buildup phase, and 0.5% during the maintenance phase, in a large, multicenter trial of over 840 subjects. Of the 280 reactions, one-third required treatment with oral antihistamines and six required epinephrine [14]. In addition to these findings, several meta-analyses estimate the overall rate of a SAR with VIT to be between 5.8 and 28.87% with an increased risk associated with the use of HB venom [15–17].

When compared to AIT used to treated environmental allergies, the risk associated with VIT is considerably lower. In a study of 454 subjects treated with inhalant AIT or VIT, those that received VIT had the lowest risk of a SAR (12%) compared to those treated for rhinitis and/or asthma (35%–79%) [18]. These data were confirmed in a prospective multicenter study involving over 1000 subjects treated with inhalant AIT or VIT. Subjects who received wasp VIT were more likely to reach maintenance dosing and less likely to have an adverse reaction, including a SAR, than those receiving AIT for cat (OR, 4.08), dust mite (OR, 2.78), birch tree (OR, 1.62), or Timothy-grass (OR, 3.61) [19].

There are few studies evaluating the safety of IFA WBE treatment for IFA hypersensitivity. La Shell et al. demonstrated that SARs occurred in 9.1% of subjects and 0.4% of injections in a case cohort study which retrospectively reviewed 77 subjects with IFA allergy. Seventy-five percent of these reactions were mild (grade 1, generalized urticaria, upper respiratory symptoms, or both), using the grading system as recommended by Mueller [20], with no grade 3 reactions (skin and/or gastrointestinal symptoms with bronchospasm, laryngeal edema, and/or shock) reported. Subjects who experienced a SAR during skin testing or a LLR following WBE injection were more likely to have a SAR associated with future IFA WBE immunotherapy (OR, 4.75 and OR, 34.5, respectively) [21]. In a study of 59 subjects treated with IFA WBE immunotherapy, only three (5.2%) experienced a SAR, all of which were considered mild. Subjects were randomized to receive twice daily oral H_1/H_2 antihistamines and corticosteroids versus placebo initiated 2 days prior to the protocol, but such pretreatment did not reduce the risk of a SAR [22].

Rush and Ultra-Rush Immunotherapy

Immunotherapy protocols that achieve maintenance dosing in a shorter time are attractive alternatives to conventional VIT, which takes several months. Although effective, these protocols are associated with more adverse reactions [14]. In a study of 118 subjects treated with rush VIT, achieving maintenance in 5 days, SARs occurred in 15.2% with Mueller grade 3 reactions occurring in 3.4% and Mueller grade 4 reactions (cardiovascular and/or respiratory collapse) in 2.5% [23]. A similar SAR rate was reported by Wenzel et al. in a retrospective study of 178 subjects utilizing a 2-day rush VIT protocol. The overall rate of SAR was 17.9% with Mueller grade 3 reactions occurring in 3.4% and Mueller grade 4 in 2.2% of subjects [24]. Goldberg et al. found that SARs occurred in 29.6% of subjects treated with a 3-day rush HB protocol; however, cost was only 41% of a conventional protocol. Eighty-five percent of treated subjects were able to reach maintenance despite SARs [25]. Other authors have found that SARs occur with less frequency. In a study by Sturm et al. 101 subjects underwent a 7-day rush VIT protocol and SARs were seen in 6.9% including 0.47% of all injections [26].

When considering ultra-rush protocols, the rate of SARs is similar. Utilizing a 210 min, 1-day, VIT protocol in 51 children and 207 adults, the rate of SARs was 10.8% and 11.2%, respectively [27]. Roll et al. found the rate of SAR to be 17.5%, although no life-threatening reactions were reported and no epinephrine was required, in a similar protocol lasting 4 h [28]. In a direct comparison of conventional (15 weeks) versus rush (5 days) and ultra-rush (180 min), the rate of any adverse reaction was similar (3.99% conventional, 3.7% rush, and 1.99% ultra-rush). SARs were associated with 0.56% of conventional and 0.9% of rush doses, while no SARs were seen with ultra-rush [29].

Rush protocols also appear to be relatively well tolerated in subjects with IFA hypersensitivity. Arseneau et al. determined that SARs occurred in 24.3% of subjects utilizing a 1-day rush protocol, although this rate decreased to 9.5% in those premedicated with H_1/H_2 antihistamines and corticosteroids 2 days prior to the protocol [30]. Tankersley et al. found that SARs occurred in only 5.2% of 87 subjects treated with a 2-day rush IFA protocol. The reactions that occurred were mild, and there was no significant difference between those who received or did not receive pretreatment medications [22].

Mast Cell Activation Disorders and the Importance of Baseline Serum Tryptase

There is an association between mast cell activation disorders (MCADs), i.e., systemic mastocytosis (SM), monoclonal mast cell activation syndrome (MMAS), and mast cell activation syndrome (MCAS), and life-threatening allergic reactions following Hymenoptera stings. In these subjects, lifelong VIT is usually recommended to prevent potentially fatal reactions following future stings. During VIT, the risk of adverse reactions, including SARs, appears higher than in subjects without MCADs. In a study of 21 subjects with SM treated with VIT, six (29%) experienced a SAR leading two subjects to discontinue therapy [31]. SARs occurred in 20.5% of all subjects in a 2009 review of case reports and retrospective studies totaling 117 subjects with MCADs [32]. Several authors have demonstrated a lower rate of SAR. Bonadonna et al. studied 16 Hymenoptera-allergic subjects with SM and only two experienced a SAR during VIT, one with generalized pruritus alone and the other generalized urticaria [33]. Similar results were reported in 84 subjects with SM and Hymenoptera venom allergy. Subjects were treated with either conventional (15 weeks) or modified (7 weeks) rush VIT, and four (4.7%) experienced a SAR with no significant difference found between the protocols [34].

Baseline serum tryptase concentration (BTC) may also be important to determine in Hymenoptera allergic subjects without MCADs. Not only can an elevated

BTC (> 11.4 μg/L) increase the risk of a SAR following a Hymenoptera sting, it is also associated with increased risk during VIT [35, 36]. The frequency of a SAR requiring emergent treatment increased with BTC in a prospective study of 680 HB or vespid allergic subjects. An increase in BTC from 4.21 to 20 μg/L increased the risk of a severe SAR by a factor of 3.75. Risk was even higher in those receiving vespid VIT (OR, 2.33, log-linear association; 95% CI, 1.28–4.26) [37]. Stoevesandt et al. also found that elevated BTC increased the risk of moderate to severe reactions during VIT compared to those with normal levels [13]. In Hymenoptera allergic children, evaluation of BTC is a useful predictor of SARs during VIT. For example, in 19 children ages 5–17, BTC > 7.75 μg/L was associated with a higher risk of a life-threatening SAR during the VIT buildup phase, while those whose levels were 4.52 μg/L experienced no adverse reactions, including SARs [38]. Based on these results, determination of BTC should be considered in subjects with Hymenoptera allergy, and some may require additional work-up to rule out an underlying MCAD.

Medication use during VIT

Cardiovascular medications, including β-blocking agents and possibly angiotensin-converting enzyme (ACE) inhibitors, are considered relative contraindications during VIT. β-Blocker use can require significantly higher doses of epinephrine needed to treat a SAR; complications such as bradycardia and bronchoconstriction also may occur [39, 40]. ACE inhibitors have been associated with the development of hypotension and SAR when continued during VIT [41, 42]. Despite these concerns, several studies suggest that both classes of medications may be safe to continue during such therapy. In a study of 1389 Hymenoptera allergic subjects who were started on VIT, a total of 44 were treated with β-blockers. Four subjects continued the medication during both buildup and maintenance, while nine had the medication restarted once maintenance was reached; twelve subjects had β-blockers newly started during VIT. SARs were reported in three (12%) compared to 23 (16.7%) subjects who did not take a β-blocker, and none were considered severe or required cessation of treatment [43]. Ten percent of 680 subjects continued some type of cardiovascular medication during VIT, including β-blockers and ACE inhibitors, in the previously described study by Rueff et al. Neither of these medications was associated with an increased risk of a SAR nor a need for epinephrine [37]. Stoevesandt et al. studied 740 Hymenoptera allergic subjects of which 190 continued their use of ACE inhibitors (11.6%) or β-blockers (3%) during VIT buildup. Neither medication increased the risk of a SAR; in fact, both medications were associated with a trend toward lower risk [44]. These findings were confirmed in a follow-up study of 225 subjects, of which 125 continued ACE inhibitors (31.6%)

and/or β-blockers (17.8%) during the maintenance phase of VIT. SARs occurred with less frequency in subjects taking cardiovascular medications than in those who did not [45]. These studies highlight that cardiovascular medications may be safely continued during VIT. Current guidelines, however, recommend that both medications be discontinued, if possible, during VIT [46]. In subjects taking ACE inhibitors, angiotensin receptor blockers (ARBs) might be a suitable alternative [47]. In cases when β-blockers cannot be withheld, caution and close monitoring during VIT are recommended.

Autoimmune Complications

In addition to local and systemic reactions, other kinds of adverse reactions have been reported during VIT. Serum sickness has been described as an uncommon complication following an insect sting, raising concern that a similar reaction could occur during VIT [48–50]. The onset of symptoms and laboratory findings (C1q and Raji cell analysis) suggestive of serum sickness was assessed in 30 adult and 15 pediatric subjects who underwent VIT for up to 29 months. None demonstrated clinical symptoms or a positive C1q, but four had positive Raji cell analysis. Subsequent evaluation revealed that these antibodies were positive prior to VIT, and no change in the Raji cell titer occurred during the 12 h following VIT, suggesting that such therapy did not increase the risk of developing immune complex disease [51]. Despite these data, there are reports documenting fever, malaise, headache, and joint pain beginning 4–6 h after VIT and lasting up to several days [52].

Concern for developing autoimmune diseases following AIT also has been addressed. There are several case reports which document the onset of rheumatoid arthritis, Sjogren syndrome, and multiple sclerosis following AIT [53–55]. Although it is unclear whether there is a cause and effect, and the mechanism is unknown, there is a proposed link between aluminum hydroxide-containing adjuvants and the promotion of autoimmunity [56]. Despite these concerns, several studies suggest AIT is safe, even in subjects with preexisting autoimmune diseases. Over 18,000 Danish citizens treated with AIT, including VIT, were compared to more than 400,000 citizens who received conventional treatments. AIT was associated with a lower risk of developing autoimmune diseases than conventional treatments (hazard ratio, 0.86; 95% CI, 0.74–0.99) [57]. In a case report of a patient with systemic lupus erythematosus treated with mixed vespid VIT, no exacerbation of the underlying disease occurred, even after 1 year of treatment [58]. Caution should be exercised before starting VIT and weighed against the risk/benefits in those at risk for these disorders. If VIT is initiated, they should be closely monitored for development or worsening of the underlying autoimmune disease.

Considerations in Children and Pregnancy

Added precaution should be exercised with populations that may be at higher risk for adverse reactions with VIT. Children, especially those under the age of 5 years, are at higher risk due to their potential inability to recognize and report symptoms of a SAR. VIT appears to be efficacious in children of all ages with a similar safety profile as seen in adults [59]. Konstantinou et al. demonstrated a low risk of any side effects in 53 Hymenoptera-allergic children, median age 9.5 years. They were treated with VIT for 2 years, and 51 tolerated the protocol without a SAR [60]. Ultra-rush protocols also appear to be safe. Kohli-Wiesner et al. utilized a 210 minute ultra-rush protocol in 94 Hymenoptera-allergic children, 4–15 years of age. SARs were seen in 16% with 5% of these experiencing a Mueller grade 3 reaction [61]. Similar work by Steiss et al. in 43 children, ages 4–18 years, treated with modified ultra-rush VIT demonstrated that 58.1% experienced no adverse reaction of any kind; LLRs were seen in 25.6% and no SARs were noted [62].

Pregnant women that develop an allergic reaction triggered by a Hymenoptera sting are at risk for complications including premature delivery, fetal abnormalities, and even death of the mother and/or fetus. Initiation of VIT during pregnancy also may be associated with an increased risk of complications. In addition to the risks of a local and SAR observed in nonpregnant subjects, there are reports of uterine contractions occurring during treatment with HB venom [63, 64]. Therefore, VIT should not be initiated during pregnancy, unless justifiable [46]. An example may be a beekeeper or a family member of a beekeeper. Continuation of VIT is safe for women who have reached maintenance prior to becoming pregnant. Twenty-six VIT-treated women experiencing 43 pregnancies were followed until termination. Thirty-six pregnancies terminated with normal outcomes; two adverse reactions occurred, neither of which required treatment. One pregnancy was associated with multiple congenital birth defects of unknown etiology not felt to be secondary to the VIT [65]. Similar results are seen during inhalant AIT, providing further evidence that VIT is safe to continue during pregnancy [66].

Reducing the Risk of Adverse Events

Factors exist that increase the risk of SARs during skin testing and treatment for Hymenoptera venom allergy and include female sex, use of HB or yellow jacket venoms, and elevated BTC or a history of MCAD. Other factors, such as utilizing rush or ultra-rush protocols, may also increase the risk. Continuation of certain cardiovascular medications, specifically β-blockers and ACE inhibitors, also may increase the risk of SARs, but this is controversial. Recognizing these factors and appropriate adjustments in the treatment plan, when indicated, is important for optimal safety with VIT.

The use of premedications has been evaluated to determine if such treatment decreases the incidence of VIT-associated SARs. One-hundred and twenty-one subjects with Hymenoptera allergy were randomized to one of three pretreatment groups: terfenadine (no longer FDA approved in the USA) plus ranitidine, terfenadine alone, or placebo. Subjects who received terfenadine were less likely to discontinue therapy due to a SAR and had less localized erythema, edema, and pruritus compared to placebo [67]. In a similar study, Wohrl et al. randomized 15 Hymenoptera-allergic subjects to pretreatment with montelukast versus desloratadine or placebo. Subjects received the premedication regimen 1 h prior to dosing on each day of a 5-day rush VIT protocol. Montelukast, but not desloratadine, decreased the development of LLRs during the protocol. SARs were seen in two subjects, but the authors were unable to determine if the use of either montelukast or desloratadine affected the SAR rate [68]. Pretreatment with H_1/H_2 antihistamines and corticosteroids in IFA allergy has yielded conflicting results [21, 30]. Omalizumab, a humanized antibody which binds free IgE and prevents binding to the high affinity IgE receptor (FcεR1), also has been evaluated in Hymenoptera allergy. Studies document a reduced rate of SARs, improved tolerance in subjects who discontinue VIT due to SARs, as well as improved VIT safety in subjects with MCADs [69–73].

Comparison between the types of extracts and their effects on SAR rates also are reported. Depot extracts, modified with aluminum hydroxide, have been hypothesized to cause fewer SARs than aqueous extracts [74, 75]. Rueff et al. randomized 56 HB allergic subjects to rush VIT utilizing aqueous extracts versus an aluminum hydroxide depot preparation. Aqueous extracts were associated with a higher rate of both LLRs and SARs compared to aluminum hydroxide-based extracts [76]. Purified extracts, which have undergone filtration to remove low molecular weight compounds <1000 diopters, are also associated with fewer side effects than non-purified extracts [18]. For example, purified extracts were associated with a lower risk of severe, localized reactions (9%) and SARs (0.9% of injections, 11% of subjects) versus non-purified extracts (local, 24%; SAR, 2.7% of injections and 30% of subjects) in a study of 94 Hymenoptera-allergic subjects randomized to receive purified versus non-purified HB or yellow jacket VIT [77].

Sublingual immunotherapy (SLIT) also has been evaluated as a safer route of therapy for Hymenoptera allergy. Only two subjects experienced mild, local reactions during buildup with none seen during maintenance dosing in a pilot study of 21 subjects who received wasp SLIT VIT for a total of 24 months [78]. Despite these encouraging results, questions remain regarding the efficacy of SLIT for Hymenoptera allergy; additional studies are needed [79–81].

The most important aspect of determining a sting reaction is to perform a detailed history of the sting(s). The medical history and subject risk factors also are important prior to venom testing as is careful selection of the venom extract and treatment protocol utilized. Physicians and other healthcare professionals must be trained to recognize, assess, and treat SARs when they occur. Correct identification and confirmation of each subject tested and prescribed VIT are of paramount

importance. Proper technique should be utilized during skin testing and VIT administration and SPT should always be performed prior to ID tests. When VIT is initiated, verification of the correct vaccine for an individual subject is mandatory. Subjects receiving such therapy should receive instructions about the potential signs and symptoms of a SAR and delayed-onset reaction which can occur after discharge, and they should be monitored for at least 30 minutes following their VIT injection. Appropriate emergency medications, including epinephrine, antihistamines, and corticosteroids, should be readily available in the clinic setting where testing and VIT are performed. Other supportive measures such as oxygen, equipment for intravenous access, inhaled β-agonists, and vital sign assessment devices should also be available.

The use of self-injectable epinephrine devices for subjects treated with VIT remains a controversial subject. According to current guidelines, all subjects with a history of a SAR triggered by any allergen, including venom allergens, should be prescribed a self-injectable epinephrine device with instructions for use [82]. Such recommendations focus on those subjects with risk factors such as uncontrolled asthma, comorbidities that could increase the risk of a SAR, and those who are at high risk of exposure to the allergen to which they are allergic. Variable characteristics of affected subjects, the nature of the allergic reaction, and uncertainty regarding the proper indication for appropriate use of an auto-injector make the prescribing of this medication more complicated [83]. Utilization of these devices also is associated with significant economic impact. The estimated cost for the use of prophylactic self-injectable epinephrine was $469,459 per year of life saved and $6,882,470 per death prevented in a 2007 cost analysis using Markov modeling of a SAR following a Hymenoptera sting in a 6-year-old child. Based on these results, the authors concluded that the risk of fatality from Hymenoptera sting needed to be above 2 per 100,000 persons at risk to be cost-effective, which is higher than what occurs in a practice setting [84]. Given the cost associated with use of these devices, the extremely low risk of mortality with VIT, and that most reactions happen within 30 min of the injection, self-injectable epinephrine devices may not be necessary in most subjects on VIT. For those at high risk, these devices would likely be more beneficial.

Conclusions

Local and systemic reactions occur with both skin testing and VIT, although the overall rate is low. There are a variety of factors that increase the risk of SAR which need attention during evaluation and treatment of Hymenoptera allergy. Baseline serum tryptase concentrations may provide additional risk stratification and should be considered in all subjects. A variety of strategies exists to reduce risk and should be implemented during the management of subjects with Hymenoptera venom allergy (Fig. 8.2).

Risk factors	Risk reduction strategy
Patient	
Pregnancy	• Do not initiate VIT during pregnancy unless justifiable • Safe to continue maintenance VIT during pregnancy
MCAD/elevated BTC	• Consider BTC concentration in subjects with SARs following Hymenoptera sting
Autoimmune diseases	• VIT appears safe to start/continue • Monitor for worsening of underlying disease during treatment
Cardiovascular medications β-blockers/AC inhibitors	• May be safe to continue during VIT • Consider alternative medications such as ARBs when possible • Close monitoring if medications are continued
Female sex	• Note
Age < 5 years	• Note
Skin testing	• Verification of correct subject and venoms to be utilized during testing • Ensure underlying atopic diseases (asthma, atopic dermatitis, etc.) are controlled prior to testing • Proper technique: o Always perform SPT prior to ID testing
VIT/IFA WBE	
Use of honeybee venom Use of yellow jacket venom MCAD/elevated BTC Rush/ultra-rush protocols	• Verification of correct extract/subject prior to dosing • 30 minute observation after dosing • Ensure underlying atopic diseases (asthma, atopic dermatitis, etc.) are controlled prior to dosing • Pretreatment with H1/H2 antihistamines, montelukast, and/or corticosteroids may reduce risk • Consider depot rather than aqueous extracts, where available • Prescribe a self-injectable epinephrine device in high-risk subjects • Consider omalizumab in subjects who cannot tolerate VIT/WBE due to SARs or in those with MCADs • Consider “slower” protocols in subjects who experience SARs during rush/ultra-rush VIT

Fig. 8.2 Adverse reactions associated with skin testing and VIT with recommendations for risk reduction

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Chapter 9
Duration of Venom Immunotherapy

David B.K. Golden

The duration of venom immunotherapy (VIT) has been a concern for patients and clinicians since VIT was first introduced. Despite numerous studies, the answer remains somewhat uncertain, owing largely to the heterogeneity of the patient population and of the allergic condition itself. It is also very difficult and costly to do the long-term studies that are needed. There would be a need to perform sting challenges in large numbers of patients, which is possible in very few centers in the USA or Europe (where it is considered unethical in some countries). Since VIT was introduced in 1979, the product package insert states that VIT is recommended indefinitely. Based on this labeling, clinicians would be justified to make this recommendation to any patient who is treated with VIT. However, there is adequate published evidence to support discontinuing VIT in the majority of patients, based on the identification of high-risk and low-risk patients [1, 2]. However, the decision to stop VIT is also made difficult by the fact that there is no guarantee of safety for any patient who discontinues VIT.

There are no new research observations in recent years, but there has been a gradual evolution of the consensus on appropriate recommendations to different kinds of patients regarding whether and when to discontinue VIT. This can depend on a number of clinical characteristics, as well as quality of life and medicolegal concerns.

When VIT is discontinued, there will always be some residual risk of reaction. The chance (frequency) of reaction, and the severity of any reaction (should it occur), can be statistically described but remain difficult to predict in any individual

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D.B.K. Golden, M.D.
Johns Hopkins University, 7939 Honeygo Blvd. #219, Baltimore, MD 21236, USA
e-mail: dgolden1@jhmi.edu

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patient. There is considerable variation in the judgment of the clinician and the patient regarding the degree of tolerable risk. The spectrum of risk (chance of a systemic reaction to a sting) in untreated patients ranges from normal (1–2% in the general population) to very low risk (<5% in large local reactors), low risk (10–15% in mild systemic reactors), moderate risk (20–30% in moderate systemic reactors), and high risk (40–75% in severe systemic reactors). There has been no clear agreement on how much risk is "too high" in the case of sting anaphylaxis, but clearly the best case scenario would be to stop VIT when the risk returns to "normal." The risk of anaphylaxis is near normal during VIT but is at least somewhat greater when VIT is discontinued. The risk does not change significantly during 10 years of observation after stopping VIT [3–6].

In the case of inhalant allergen immunotherapy, the decision is often based on the observed efficacy as well as the duration of treatment (usually 3–5 years), although relapses certainly occur and may require changes in medications or resumption of immunotherapy. Venom immunotherapy has been held to a different standard, presumably due to the potentially life-threatening nature of the disease. This potentially lethal outcome often makes physicians and patients unwilling to accept a risk of recurrence that they might accept with ragweed immunotherapy.

To Stop or Not to Stop

During VIT, the risk of systemic reaction to a sting is 2–20% depending on the insect species and other factors. Reactions to a sting during VIT are generally much milder than the pre-VIT reaction. When VIT is stopped based on published criteria, there is less than 10% chance of systemic reaction to a sting, almost all of which are milder than the pre-VIT reaction. With the identification of "high-risk factors," it has become possible to stop VIT in only "low-risk" patients [2, 7]. However, even in low-risk patients, the chance of a systemic reaction to a future sting does not return to "normal." Therefore, one of the options would be to not stop VIT. This would keep the risk at the lowest possible level in sensitized individuals. Given all the available information, the choice of many patients is to continue VIT because it is easy and safe and makes them feel the most secure (which is quite true). Although stopping VIT and carrying an epinephrine autoinjector might seem just as good to the physician, there is an associated burden that can cause impairment of quality of life [8, 9].

To assist the patient and physician in making this decision, there are numerous studies that provide useful observations on which factors are most useful in predicting the outcome when VIT is discontinued. In reviewing the available evidence, one must keep in mind the two elements of risk: frequency and severity. In terms of relative risk, a 50% chance of a very mild reaction might be less worrisome than a 2% risk of a life-threatening reaction.

Length of VIT Treatment

One approach is to define a specific length of time such as 3 years or 5 years after which stopping treatment would not lead to an unacceptable frequency of significant systemic reactions to subsequent stings. When VIT was stopped after 1–2 years, there was more than 20% frequency of systemic reactions to subsequent stings [10, 11]. Some investigators initially reported success in stopping treatment after 3 years [6, 12, 13]. However the actual duration of VIT in those studies was "3 or more" years with a mean of more than 4 years. Also, those studies included other criteria such as negative RAST and negative sting challenge during therapy. These criteria make it impractical or unlikely that a patient could stop VIT after 3 years because very few patients have a negative serum IgE for venom after just 3 years and sting challenge cannot be performed outside of a few research centers.

When VIT was discontinued after more than 4 years of treatment (mean of 6 years), there was a 10% incidence of systemic reactions to subsequent stings, almost all of which were much milder than the pre-VIT sting reaction [5, 14, 15]. Treatment for 5 years gave better immunologic and clinical outcomes than 3 years [5, 15]. These studies helped to identify high-risk factors that are associated with a greater chance of relapse after stopping VIT (discussed in detail below). Some studies have continued to suggest that shorter duration of treatment can give long-term outcomes as good as a longer duration of VIT, but all of those reports were underpowered and therefore unlikely to detect the difference they purported to examine. There continues to be some agreement that 3 years of VIT might suffice for certain patients with lower overall risk, such as those with milder history and younger individuals, particularly children [6, 16]. In a long-term follow-up of children who had been off VIT for 10–15 years, only 5% had a systemic reaction to a sting, and none were severe [16]. In that study, the mean duration of VIT was 3.5 years.

The outcome in children has been quite similar to that of adults. Studies in children who stopped therapy after 5 years also showed a very low risk of reaction to subsequent stings [17, 18]. Long-term studies showed sustained tolerance of stings in children who completed 5 years of VIT. Children who were surveyed 5–15 years after stopping VIT had a 5% frequency of systemic reaction to a sting [16].

Unlike the venom skin tests and IgE levels (which decline steadily in the 5–10 years after stopping VIT), the chance of a systemic reaction to a sting did not diminish during up to 13 years of observation [4, 6]. Therefore, patients who got stung more frequently during these years of observation had more chance that at least one of the stings would cause a systemic reaction (17% overall in the study) [4]. As is the case in untreated patients, the occurrence of a sting reaction after discontinuing VIT is unpredictable and can occur with one sting and not another.

Immunologic Changes or Other Test Procedures

Immunologic observations of maintenance venom immunotherapy showed that the venom-specific IgE levels, which rise during the initial months of therapy, decline gradually during years of treatment, generally falling to low levels by the fourth or fifth year on treatment (but rarely becoming undetectable) [10]. After a mean of 6 years of VIT, venom skin tests remain positive in about 75% of patients but show decreased sensitivity in almost all of the patients. After stopping treatment, venom sensitivity continues to decline even after challenge stings, becoming negative in 70% of patients tested several years after stopping treatment. Some patients who have had sting reactions after discontinuing VIT showed no decline in venom sensitivity [4]. However, this occurs in so few patients after 5 years or more of VIT that it has not been possible to demonstrate a significant increase in the chance of relapse (systemic reaction to a sting after VIT is stopped) [5].

The venom-specific IgG antibody levels are maintained at therapeutic levels during this treatment but decline to pretreatment levels within 6–12 months of stopping VIT [18]. The level of venom IgG correlates with clinical protection during the first 3 years of VIT, but tolerance of stings is independent of the IgG level beyond the first 3 years and has no predictive value after stopping VIT [19].

History of Pre-VIT Sting Reactions

The severity of the sting reaction before venom immunotherapy has been reported as perhaps the most important determinant of the risk of reaction after stopping therapy [6, 15]. Some studies found no difference between reactors and nonreactors (to a sting post-VIT) regarding severity of the systemic reaction before treatment [13, 14], while others found a higher frequency of reaction to stings in patients who had more severe reactions pre-VIT [5, 6, 15]. Whether or not the frequency of systemic reaction is higher, the chance of the reaction being severe has been consistently reported to be higher in those with a history of severe reaction pre-VIT [2, 3, 6]. This leads to the conclusion that patients who had life-threatening reactions should continue venom immunotherapy indefinitely.

This statement presents a dilemma to the patient and the clinician. How much risk is too much risk? How severe does it have to be, to be an “extreme” or “life-threatening” or “near-fatal” systemic reaction? There have been numerous grading schemes for anaphylactic reactions, but some patients are more frightened by severe generalized urticaria and facial swelling (usually considered to be “mild” cutaneous systemic reactions), than by light-headedness (mild hypotension) or a lump in the throat (mild airway edema) (usually considered to be moderate). Respiratory distress or near unconsciousness is generally considered severe (with or without urticaria). Severe reactions, especially when they lead to cyanosis or unconsciousness, are usually considered “life-threatening” or “near fatal.”

There remains a question of whether VIT could be discontinued after 3 years in "low-risk" patients, such as those with a history of relatively mild systemic reactions to stings. This seems reasonable in children [16], but very few adults with mild reactions have been studied, so the safety of stopping VIT after 3 years in such patients is uncertain.

Tryptase/Mastocytosis

Patients with elevated baseline serum tryptase (with or without mastocytosis) have an increased risk of severe anaphylaxis to stings, systemic reactions to VIT, and treatment failure (reaction to stings during VIT) [20–22]. This risk is also present if they stop VIT, and even fatal reactions to stings have been reported in such patients [23, 24]. Elevated baseline tryptase is not as unusual as previously thought, occurring in about 10% of patients with a history of systemic reactions to stings and in up to 25% of those who had hypotension after a sting [21, 25]. Mastocytosis is reported in 1–5% of patients with insect sting allergy, and conversely, insect sting is the most common cause of anaphylaxis in patients with mastocytosis, occurring in 20–30% of cases [26]. Despite the greater risk of reactions to treatment, VIT is recommended in patients with mastocytosis because there is evidence that it significantly decreases the risk of reaction to stings [21, 27–29]. In these patients, VIT should be continued indefinitely [30].

Insect Species

Honeybee venom immunotherapy is associated with a higher frequency of systemic reactions to the venom injections and a higher incidence of treatment failures [31–33]. It is therefore not surprising that honeybee-sensitive patients also have a greater chance of relapse when therapy is discontinued [5, 34, 35].

Clinical protection may not be as well preserved in patients who discontinue immunotherapy for allergy to imported fire ants [36, 37]. This may be related in part to frequent exposure and a high attack rate for fire ant. It is not known whether the outcome after stopping VIT might differ in patients with *Polistes* wasp sting allergy.

Systemic Reactions During VIT

Treatment failure (systemic reaction to a sting during VIT) can occur in about 5% of patients on vespid venom therapy and in more than 15% of those receiving honeybee venom. Systemic reactions to venom injections occur in 5–10% of patients,

mainly during the buildup phase of treatment. These reactions during VIT are generally much milder than the pre-VIT reactions. Most of these patients subsequently do well and have no further reaction to venom injections or stings. Two studies have examined whether such patients can safely discontinue VIT after 5 years. They found that patients who have a systemic reaction during VIT, either to a venom injection or a sting, have a significantly higher chance of relapse after discontinuing VIT [13, 38]. Patients who have systemic reactions to stings during VIT can achieve more reliable protection with increased doses of VIT, but it is not known whether this mitigates the increased risk of stopping treatment several years later in these patients [39].

Medical Conditions and Medications

The morbidity and mortality of insect sting anaphylaxis increase with age [40, 41]. It is assumed that the greater mortality from sting anaphylaxis in older individuals is due at least in part to their greater frequency of underlying cardiac and vascular conditions that may predispose to a poor outcome in anaphylaxis [41]. These factors should be taken into account when considering whether to discontinue VIT.

The chance of a severe reaction is increased by some medications, notably angiotensin-converting enzyme inhibitors (ACEI) and beta-adrenergic blocking agents (BB). This risk seems to be lessened during VIT [42, 43], but it is not known whether these medications can affect the outcome of a sting after VIT is discontinued. In patients beginning VIT, the risk of stopping these medications may be greater than the risk associated with continuing them during VIT [41, 44]. The risk of withholding VIT is also considered to be greater than that of administering VIT while on these medications. However, because the chance of severe reaction to a sting is quite low after stopping VIT, it is not known whether it would be justified to continue VIT just because of concern about possible sting reactions after stopping VIT in patients taking these medications.

Individual/Social Factors

The frequency of exposure to stinging insects is a risk factor or recurrence of systemic reactions after stopping VIT [4, 5]. The estimated chance of reaction is 10% for each sting, so if the patient sustains several stings in the years after stopping VIT, the cumulative chance of a systemic reaction is about 17% [4, 5]. Prior to VIT, sequential stings in the same summer are likely to increase venom-specific IgE levels, and there is a higher chance of systemic reaction on the second or third sting of the summer [14]. Whether this is also true after stopping VIT is not known. However, patients who have repeated stings over 4–13 years of observation after

stopping VIT have a higher cumulative risk of reaction [3, 4]. It has been observed that the reaction to any given sting is unpredictable. Some patients have had no reaction to a sting only to have a systemic reaction the following year [4–6]. Therefore, an uneventful sting is not a guarantee of safety to future stings. Patients who have frequent occupational or avocational exposure to stings, and therefore a higher chance of being stung more than once over a period of years, may consider the relative benefit of remaining on VIT.

The age of the patient may be a consideration in discontinuing VIT. As described above, older individuals are known to have a greater risk of fatal reactions to stings and may have medical conditions that could complicate anaphylaxis. Children are thought to achieve tolerance earlier than adults and might be able to safely stop VIT after 3½ years [16]. Children have a lower risk for recurrent SR than adults after discontinuing VIT [5, 12, 13, 16, 45]. Lerch et al. reported that SR occurred in 8.3% of children and 13.1% of adults [5]. In long-term studies of patients 10–15 years after stopping VIT, Golden et al. found that 5% of children had SR compared to 16.7% in adults [16].

Insect sting allergy causes significant impairment in the quality of life, and this is greatly improved with VIT (but not necessarily with prescription of an epinephrine injector) [8]. This is even true in those with a history of mild systemic reactions [46]. Many patients express anxiety about the chance of a reaction after stopping VIT, despite our reassurance that the chance of a severe reaction is probably less than 2% in patients who have no high-risk factors. Because patients who continue VIT can usually be treated safely and effectively at 3-month intervals, the option to continue treatment four times a year may provide great comfort and security to such patients and may be considered for quality of life reasons.

Long-Term Outcome

The early studies of discontinuing VIT showed progressive disappearance of the venom IgE (by skin test or serum IgE) and the lack of re-sensitization by intervening stings [5, 13, 16]. Those studies also reported that the risk of reaction seemed to decline with time after discontinuation. However, extended observations suggest that the risk does not disappear with time [4–6]. Some patients had systemic reactions to stings more than 10 years after stopping treatment. A study of 113 patients who stopped VIT after a mean of 6 years of treatment showed that the frequency of systemic reaction to a sting was 10% for stings occurring in the first 4 years off therapy and was also 10% for stings occurring 5–13 years after treatment [3]. This is becoming a question of increasing importance, because there is a growing number of patients who are on extended VIT due to either high-risk factors or quality of life concerns. We now have to ask “how long is long enough?” Although there are more and more patients who have been on VIT for 15–25 years, there are no data on the outcome in those patients if they stop treatment.

Summary

The decision to discontinue venom immunotherapy must take into account the available published observations, the natural history of the disease, the individual patient's pattern of reaction, and the judgment of both the allergist and the patient. There are several specific factors that are associated with a greater risk of relapse (see Table 9.1), but all patients must be aware that they will continue to have a 10% chance of having a systemic reaction to a future sting, even if venom skin tests become negative and even if there is no reaction to one sting after stopping treatment. This means that there is no guarantee of safety for any patient who discontinues VIT. Although it is said that majority of patients can discontinue treatment after 5 years, the various risk factors and caveats mean that some 30–50% of patients on VIT could probably be excluded from stopping VIT after 5 years.

Candidates for indefinite treatment include patients who have a history of very severe reaction to previous stings, elevated basal serum tryptase, systemic reaction during VIT (to injection or sting), honeybee anaphylaxis, or frequent exposure. Candidates for extended (>5 years) treatment also include patients who have had no decrease in venom IgE or skin tests, have underlying cardiovascular or respiratory disease, use of ACE inhibitors or beta-blockers, or have had less than 5 years of maintenance VIT.

Patients who have decided to discontinue VIT will benefit from counseling about the risk factors for relapse and the relative chance of a systemic reaction (and the chance of it being severe enough to use epinephrine autoinjector). Patients should be aware that subsequent sting reactions are unpredictable and can occur after one or more stings that cause no reaction. The chance of reaction is greater with more frequent exposure. Patients and physicians should consider whether the cost and burden of carrying an epinephrine autoinjector are justified by the benefit to the patient. Ultimately, the decision must be made after thorough discussion between the physician and patient to identify the needs and preferences of the individual. One patient might feel that a 10% chance of reaction is quite acceptable, and another might feel that even 1% chance of a severe reaction is unacceptable.

Table 9.1 Candidates for indefinite or extended (>5 years) treatment with venom immunotherapy

Candidates for indefinite treatment:
• Very severe reaction to previous stings
• Elevated basal serum tryptase
• Systemic reaction during VIT (to injection or sting)
• Honeybee anaphylaxis
• Frequent exposure
Candidates for extended (>5 years) treatment:
• No decrease in venom IgE or skin tests
• Underlying cardiovascular or respiratory disease
• Use of ACE inhibitors or beta-blockers
• Less than 5 years of maintenance VIT

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Part II

Chapter 10
Unique Aspects of Bee Allergy and Reactions

William R. McKenna and Tyson C. Brown

Introduction

The honeybee (Fig. 10.1) is perhaps the most recognized insect in the world. It has been used for both agricultural and medicinal purposes for hundreds of years. Perhaps because of its abundance and high recognition, it may be one of the most well-studied insects throughout history, especially from a clinical perspective. Venom from the honeybee and the resulting treatment from sting reactions, particularly allergic reactions, have unique characteristics that set it apart from other flying Hymenoptera.

This chapter will be divided into three sections, Unique Aspects of Honeybees, Venom, Diagnosis, and Treatment of Hypersensitivity, Unique Clinical Reactions to Honeybees, and Africanized Honeybees. Focus will be in reference to honeybee-specific aspects for both the European honeybee (*Apis mellifera mellifera* or *Apis mellifera ligustica*) and in light of the relatively recent clinical significance, the Africanized honeybee (*Apis mellifera scutellata*).

W.R. McKenna, M.D. (✉)
Department of Medicine, University of Texas Health Science Center San Antonio, San Antonio, TX, USA
e-mail: wrmckenna@aol.com

T.C. Brown, M.D.
Department of Medicine, Landstuhl Regional Medical Center, Landstuhl 66849, Germany
e-mail: tcbrown76@gmail.com

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Fig. 10.1 Honeybee

Unique Aspects of Honeybees, Venom, Diagnosis, and Treatment of Hypersensitivity

Epidemiology

Honeybee stings are relatively common as data suggest as many as one-third of all adults will be stung at least once in their lifetime [1–3]. Stings from a honeybee account for about one-third of all flying Hymenoptera stings, so they appear to actually be less common than Vespid stings overall, perhaps contrary to belief that honeybee stings are the most common, though there is most certainly geographical variation. No exclusive study exists looking at the prevalence of honeybee stings in children, but extracted data show that sting prevalence for all flying Hymenoptera is lower for kids than adults [4]. There is very little data looking at deaths from honeybee stings, but two studies report that most sting fatalities (at least more than 50%) are a result of honeybee stings [5, 6], implying around 20 deaths per year in the United States. Interestingly, despite fears of colony collapse disorder and other conditions adversely affecting honeybees and hives, honeybee sting rates do not appear to be decreasing [7, 8].

More concerning systemic allergic reactions to all flying Hymenoptera occur in about 3% of all adults, and honeybee stings account for roughly half of these [2, 9]. No specific study has evaluated honeybee systemic reaction rates in children, though it appears that the overall flying Hymenoptera systemic reaction rate for children is lower than for adults [10]. It has been demonstrated already that approximately 20% of all adults are sensitized to flying Hymenoptera venom, and honeybee venom sensitization accounts for about one-third of these [9, 11, 12]. One study involving children demonstrated a substantially lower sensitization rate for all flying Hymenoptera of just under 4%, but of interest was that 81% of the positives were due to honeybee [10]. It is insightful that children appear to be more often sensitized by honeybee than adults, but this may be simply explained by the outdoor environment of their play. There also appears to be geographic variation in sensitization to various honeybee venom components, which was suggested to be a result of either differences in honeybee populations or varying human genetic differences [13].

Entomological Factors

Honeybees are certainly unique compared with other flying Hymenoptera for many reasons. They thrive in colonies numbering from hundreds to tens of thousands. The colony has a queen that typically lives 1–2 years. All bees have a specific role within the colony. The bee which typically stings a human is a forager, if away from the colony, or a soldier/guard, if near the colony. Foragers have been known to stray several miles from the hive in search of nectar. Honeybees are relatively docile in nature when compared to their Vespid counterparts. One exception to this is the Africanized honeybee, discussed in more detail later.

The forager usually visits the same group of flowers daily during the warmer months (spring and summer) [14]. They learn to recognize flowers by putting together a series of complex cues involving shape, color, scent, and location. The color recognition of a flower also involves light in the ultraviolet spectrum [15, 16]. A human is not recognized as a flower despite how they might dress; this may be important to the physician when dispensing avoidance advice. However, certain other factors have been found to place a honeybee in the defensive posture, including disturbing its foraging behavior during outdoor garden work or accidentally stepping on one, wearing dark clothes, abrupt movement, mammalian breathing, and warm, dry seasons early in the evening or late in the day [15, 17]. A honeybee's defensive behavior may also be heightened by certain substances found in various cosmetic products and foods, which may mimic the defensive pheromones released by honeybees already in a defensive posture [17].

Honeybee Stinger Apparatus and Sting Mechanism

When a honeybee is agitated, it will sting the intruder. Unlike the Vespids, the honeybee nearly always leaves its stinger in the host. This is due to large barbs on the sides of the stinger apparatus. The stinger apparatus of the honeybee is seen in Fig. 10.2. It typically has three parts, a guide stylet and two barbed lancets, which intersect to form a hollow tube through which the venom flows from the sac into the victim. The barbs anchor into the skin with such firmness that the entire stinger with attached venom sac is removed from the abdomen of the honeybee as it flies away. This separation and resulting stinger apparatus denervation, called autotomy (see Fig. 10.3), triggers the lancets to rhythmically move along the stylet in reciprocating fashion driving the stinger and venom deeper into the tissue. Once detached from



Fig. 10.2 Honeybee stinger apparatus



Fig. 10.3 Stinger separation

the honeybee, the stinger's entire envenomation process appears to take about 30–60 s on average [18]. Although this has not been evaluated, many stingers are likely to be removed by the victim well before this time. Of note, when a honeybee leaves its stinger behind, the bee typically dies within hours. It has been suggested by some that the method of stinger removal by the victim may lead to a worse reaction or better outcome. However, based on one study, it may not matter if you pinch the stinger for removal or scrape/swipe it off, as neither method resulted in a larger local reaction [19].

The amount of venom injected by the honeybee is substantially greater as compared to Vespids. Where the Vespid will inject anywhere from 1–20 μg of venom, it has been concluded that a single honeybee sting will leave between 50 and 140 μg of venom on average in its victim [18, 20]. Based on these studies, it is also evident that venom amounts and even venom composition varies, often widely, between colonies, geographical location, and type of honeybee, with reported injected amounts as high as 300 μg! This variability will certainly play a role in clinical responses for allergic patients and can account, at least in part, for systemic reactions not occurring 100% of the time.

Honeybee Venom Composition and Relevance

Honeybee venom composition was described earlier in Chap. 3 in great detail. There are approximately 113 different proteins and substances that make up this complex venom [21]. As of today, there are 12 known allergens, listed in Table 10.1. Of these, there are six major allergens, Api m 1, 2, 3, 4, 5, and 10, though there may be some debate regarding Api m 4 and Api m 5.

Phospholipase A_2 (PLA_2), Api m 1, the allergen to which honeybee extract is standardized, is perhaps the most potent of all the allergens. PLA_2 is a glycoprotein that causes cell membrane disruption by hydrolyzing phospholipids, thus facilitating cell lysis and death [22]. This process, among other things, causes an elevation

Table 10.1 Known honeybee allergens. Source: WHO/IUIS Database—www.allergen.org

Allergen	Name	Size (kDa)
Api m 1	Phospholipase A2	16
Api m 2	Hyaluronidase	39
Api m 3	Acid phosphatase	43
Api m 4	Melittin	3
Api m 5	Dipeptidyl peptidase IV	100
Api m 6	?	8
Api m 7	CUB serine protease	39
Api m 8	Carboxylesterase	70
Api m 9	Serine carboxypeptidase	60
Api m 10	Icarapin variant 2, carbohydrate-rich protein	50–55
Api m 11	Major royal jelly protein	50 (deglycosylated form)
Api m 12	Vitellogenin	200

in leukotrienes resulting in significant surrounding inflammation. Of course, in allergic individuals, this local effect is in addition to the allergic process started from cross-linking of IgE receptors on mast cells and basophils. PLA_2 was initially thought to lead to specific antibody production and detection in up to 95% of allergic individuals [23], making it a good candidate for standardization. However, recently, through recombinant technology (rApi m 1), which is devoid of any cross-reacting carbohydrates or other confounding components, specific IgE were demonstrated in 72% of allergic individuals [24]. Similar recent reports have shown 58–80% sensitization among allergic individuals [25–30].

Hyaluronidase, Api m 2, is an enzyme that breaks down the matrix of connective tissue surrounding the injection site and facilitates the action and spread of other venom proteins [31]. It is somewhat related to hyaluronidase found in Vespid venom but may not have as much cross-reactivity as once thought [32]. Because specific IgE to this venom component is consistently found in 50% of allergic individuals [24, 33, 34], it was also long regarded as the second most potent honeybee venom component; however, in light of the discovery of other bee venom components, this is being challenged.

Among the flying Hymenoptera, acid phosphatase, Api m 3, seems to be found exclusively in the honeybee venom, though comprises only about 1% of the total bee venom by weight. Coincidentally, it is also found in imported fire ant venom [35, 36]. Though considered one of the major allergens because it leads to specific IgE in 37–50% of individuals, clinically, it may be one of the weaker allergens [24, 35]. Interestingly, it was recently found to be of relatively low quantity in venom used for immunotherapy, which may have clinical significance, as will be discussed later [24].

Melittin, Api m 4, accounts for up to 50% of the total honeybee venom by weight and in a similar, and perhaps more potent manner as PLA_2, has been well established to cause cell lysis of nearly every cell line by disrupting the cell membrane [37]. This

venom component is also unique to the honeybee. Like Api m 3, from an allergic perspective, this may be one of the weaker of the major honeybee allergens and elicits specific antibody in 23–50% of individuals [24, 38]. However, the cellular destruction from melittin is a very important factor in toxic reactions from mass envenomation as discussed later in the Africanized honeybee section of this chapter.

The function of dipeptidyl peptidase IV, Api m 5, is still not completely established, though it is thought to potentiate the action of the other venom components through directly converting them to active forms [39]. Api m 5 is moderately similar to its Vespid counterpart, Ves v 3, and elicits about 60% specific IgE in allergic individuals [24, 39].

Icarapin, Api m 10, has been of increasing importance since it was found to be of greater allergenic significance than once thought [40]. This component has been demonstrated recently that it may be significantly affected by the extraction process [40] and result in poorly existing quantities in extract used for immunotherapy [24]. This contradicts earlier assumptions that the honeybee venom was stable after extraction [37]. As will be discussed in more detail later, the underrepresentation for both Api m 10 and Api m 3 may play a significant role in honeybee venom's poorer immunotherapy effectiveness.

It is worthy to note that recent advances have shown that not only is icarapin a major under-recognized allergen, but has 11 variants, some of which may be uniquely recognized by specific IgE [41]. Of these 11, however, only variants 2–4 seem to have any clinical significance, though others have demonstrated specific IgE. Interestingly, variant 2 is the only protein used currently in recombinant diagnostic methods for Api m 10. There are also four variants discovered for Api m 6 as well. This is profound, as it further complicates the picture to know that there are not just underrepresented allergens in the extract but that there are variants of that same protein, many of which can lead to specific IgE.

The remaining known allergens (Api m 6, 7, 8, 9, 11, 12), listed in Table 10.1, are thought to be of little clinical significance, currently. However, this seems to be an evolving target as they all clearly lead to specific IgE and, hence, cause allergic reactions. Some have also been found to be the only venom component to which honeybee-allergic individuals make IgE! [24].

It is important to note as well that honeybee venom contains many other neurotoxins, mast cell-degranulating proteins, neurotransmitters, vasoactive substances, sugars, lipids, and pheromones. These are described in great detail both in this chapter and in Chap. 3. Many of these substances can further facilitate the allergenicity of the known honeybee venom components and/or lead to pain and possibly toxicity.

Diagnosis of Honeybee Venom Allergy

When a patient is determined to have had a systemic reaction consistent with an allergic reaction to a sting from a flying insect, the physician and the patient discuss whether to pursue further testing in order to establish a cause and a diagnosis.

Generally, testing should commence with the anticipation that a positive result will lead to a recommendation for intervention, usually immunotherapy. The recommendation to start venom allergy shots is not an easy decision because of both the time commitment and the potential risk. Unfortunately, however, this discussion is often complicated because of our imperfect methods for testing and our lack of complete understanding of the allergenic venom components, which sometimes leads to confusing results. Quite often, these cases involve honeybee venom. Although both diagnosis of venom allergy and treatment using immunotherapy have been discussed in earlier chapters, here the focus is why the honeybee complicates these discussions.

Initial diagnosis starts with recognition of the flying insect. Recent evidence supports the generally accepted notion that adults have a hard time discriminating between various flying Hymenoptera [42]. However, in this same study, it turns out that honeybee may be the exception and was correctly identified in over 90% of participants. Despite this evidence, unless the culprit insect can be expertly identified, usually by bringing in the specimen, testing should be performed to all five flying Hymenoptera, mainly due to the consequence of incorrect identification [43].

Testing to all five flying Hymenoptera, unfortunately, sometimes leads to positive sensitivities to several insects despite the surety of the patient's identification of the single culprit insect or a clear history or a single stinging event (i.e., the double-positive patient). Currently in the United States, there are two widely available methods for testing for sensitivity to honeybee venom, intracutaneous testing and serologic testing. Honeybee venom used for diagnostics and immunotherapy is extracted from the honeybee venom sac using electrostimulation techniques. Serologic testing is usually accomplished using immunoassay capture (CAP-FEIA). These testing modalities for venom sensitization have been described in detail in an earlier chapter and are standardized to a single allergen, for both honeybee (Api m 1) and Vespid (hyaluronidase, Ves v 2). However, honeybee venom is particularly problematic because it is comprised of over 100 different proteins and peptides [41] to which the body might experience a reaction, IgE mediated or otherwise, which further separates it from Vespids. The main difference is that there appear to be more clinically significant major honeybee venom components to which patients are sensitized and the venom makeup is more complex than for Vespid venoms, and this one key difference leads to a myriad of diagnostic difficulties.

The gold standard method for detecting venom sensitization is the intracutaneous test using concentrations up to 1 μg/mL for intradermal injections. Higher concentrations produce more irritant reactions and lower specificity, particularly for the honeybee venom [44, 45]. Melittin, for example, is well known to be a cause of pain and inflammation around the site of an injection but is not found in the Vespid venom. Because it makes up nearly half of the honeybee venom by weight, its contents are particularly irritating to the skin [43].

The sensitivity, based on clinical history, to intradermal testing, however, using standardized extracted honeybee venom is not unlike that of Vespids despite their differences in venom composition [24, 46]. The problem arises, though, for the double-positive patient, which occurs in up to 40% of venom-allergic patients [47,

48]. There exist four main reasons that a patient might be double-positive: truly double-positive sensitization, which is thought to be uncommon; cross-reacting IgE to honeybee and Vespid venom components (Api m 2/Ves v 2, Api m 5/Ves v 3, Api m 12/Ves v 6) [39, 49, 50]; cross-reacting IgE to cross-reacting carbohydrate determinants (CCD) found attached to various venom components in both venoms; and as of yet undetermined components/allergens.

As technology improves, recombinant venoms (via component resolution) are being evaluated for their usefulness in both diagnosis and treatment. Use of the recombinant venoms is attractive as it may eventually lead to more reliable venom concentrations for testing and treatment and perhaps one day even be more cost-effective. However, using naturally extracted venom for both honeybee and Vespid, allergy diagnosis currently has superior sensitivity [24, 46], which was thought to be a result of it containing all the venom components. As was pointed out earlier, however, some of the honeybee venom allergens (e.g., Api m 3 and Api m 10) may exist in lower quantities in these preparations.

Vespid venom seems to have a much better result when suspected venom-allergic patients were serologically tested against recombinant PLA_1, rVes v 1, and antigen 5, rVes v 5, using CAP-FEIA, with a sensitivity, based on clinical history, of 94% [51, 52]. This accuracy rivals current serologic and intracutaneous methods, based on clinical history. The same was not found with honeybee, however, when the major allergen recombinant PLA_2, rApi m 1, was used for diagnosis using CAP-FEIA, based on clinical history and skin testing, which showed a sensitivity found to be between 58 and 80% [25–30, 53]. This contradicts earlier data that PLA_2 sIgE was found in up to 95% of individuals [23] and suggests two things: that there are other confounding proteins/sugars attached to the PLA_2 (e.g., CCDs) to which IgE might be produced and that other honeybee venom allergens are of greater clinical significance than once thought. There remains debate regarding the role/significance of CCDs in double-positive patients, some suggesting they may not be clinically/diagnostically significant [30, 54] and others feel that removing them (as in recombinant venoms) will help in distinguishing the challenging double-positive patient [25, 32]. Further studies are clearly needed to understand the role of CCDs in honeybee venom allergy diagnosis.

In a recent study by Kohler et al. [24], serum was analyzed from known honeybee-allergic patients against mostly recombinant sIgE for several known/recently discovered honeybee venom allergens. They demonstrated that although standardized to Api m 1, honeybee venom contains many other major allergens that have significant clinical importance, such as Api m 3, Api m 5, and Api m 10, and were often found as the only venom components to which some patients demonstrated sIgE sensitization. In the same study, they clearly showed as well that patients receiving immunotherapy were making significantly less sIgG4 to Api m 10 and Api m 3, suggesting that there are low amounts of these allergens in therapeutic honeybee venom preparations. This was confirmed by Api m 3 and Api m 10 sIgE CAP-FEIA inhibition studies using both therapeutic and crude honeybee venom. Honeybee venom used for diagnosis also seemed to show lower amounts of Api m 3 and 10 on inhibition studies, but these differences were not statistically significant.

These authors go on to suggest that observations from this study and their prior studies seem to point to another cause of double positivity in that double-positive honeybee venom-allergic patients seem to have higher total IgE and higher amounts of sIgE to higher numbers of sIgE when compared with those of monosensitized honeybee venom-allergic patients who tend to have lower total IgE levels with lower amounts of sIgE to a lower number of sIgE to honeybee venom components. This certainly challenges the current thought of the role of CCDs and cross-reacting venom allergens. It also suggests that there may be certain population phenotypes (e.g., atopic individuals) that are more prone to double positivity, which might be an area of future study [24].

The basophil activation test (BAT), usually measuring cell surface activation of CD63 and/or CD203c in response to venom sensitization, may be of use for diagnostic purposes. It has been postulated as a possible "next step" in distinguishing the double-positive patient [53, 54], but it appears to be most useful in helping identify those patients with clear clinical reactions to honeybee (and other flying Hymenoptera) who do not demonstrate sIgE using skin testing and serologic evaluation [55]. Although it appears to be pushing toward mainstream, the BAT is still considered more of a research tool due to lack of standardized end points and its challenging technical methodology.

Specific IgE inhibition using CAP-FEIA or RAST (radioallergosorbent test) may also offer benefit when analyzing the double-positive patient and was suggested as a "next step" when evaluating these difficult patients [56]; however, this method is still used mainly in the research setting because it is time-consuming and, like BAT, is technically difficult. Testing using dialyzed venom has been found useful for yellow jackets [57] but has not been accomplished for the honeybee to the knowledge of the authors.

Treatment of the Honeybee Venom-Allergic Patient

When a patient has been determined to have a life-threatening honeybee venom allergy, it is usually recommended that they receive venom immunotherapy in an effort to induce protection to the venom of the culprit insect. As discussed above, the decision to start immunotherapy, although seemingly straightforward, is sometimes made more difficult when it involves honeybee venom. Getting the patient on immunotherapy, however, is important because chance for re-sting for both adults and children is reported to be nearly 60% [24, 58].

Honeybee venom immunotherapy has been found to be less protective than Vespid venom immunotherapy. Patients receiving a standard 100 μg maintenance dose of honeybee venom immunotherapy appear to be 77–84% protected from future sting systemic reactions [58–61] versus about 87–94% protection for other flying Hymenoptera [58, 60–62], using natural venom extracted and lyophilized via processes described in an earlier chapter. Safety of immunotherapy is also a concern. Patients receiving honeybee venom immunotherapy have at least one systemic reaction resulting from injections in 8–41% of patients versus 2–25% reported for

other flying Hymenoptera [58, 60, 63, 64]. Of course, these numbers vary and are difficult to compare due to non-standardized systemic reaction definitions and immunotherapy protocols. Nevertheless, the overall greater risk clearly trends toward honeybee venom.

One factor recently discovered that may explain the diminished efficacy for honeybee venom includes the discovery that Api m 3 and Api m 10 are either absent or found in low quantities within the allergen extract used for immunotherapy [24]. This was evidenced by low-specific IgG4 to these components in honeybee-allergic patients receiving immunotherapy and the limited inhibition of these components on antibody inhibition measurements directly of the serum. Interestingly, it was also discovered that 5% of the cohort produced specific IgE to only these two components, suggesting that this group was possibly receiving no protection at all! It is very possible that there may be other components playing a larger-than-expected role in honeybee allergy already discovered but not realized or as yet undiscovered. Another unrelated study found an association between low sIgE to rApi m 1 and severe systemic reactions, suggesting that there may be other major allergens for which the immunotherapy is not effective [65]. Though it is not known if there are efforts underway to include recombinant forms of these major honeybee allergens as additions to the natural, lyophilized venom used for immunotherapy, it should be strongly considered. It is likely that over time we will become better able to personalize treatment based on component diagnosis.

Honeybee allergy is a well-known risk factor for systemic reactions during venom immunotherapy [43], even reported in more than one-third of individuals receiving honeybee immunotherapy [60, 63]. Most studies define concerning reactions to include both subjective and objective findings. However, during a recent study, systemic reaction rates consisting of only objective findings, based on definitions from Ring and Messmer [66] and the World Allergy Organization (WAO) [67], showed much lower systemic reaction rates of 8.1% for honeybee and 2.3% for Vespid. Notably, of the five most severe reactions, four were a result of honeybee-only-allergic patients, despite only 20% of the patients being treated for honeybee allergy [64].

Attempts to stratify risk factors for systemic reactions during venom immunotherapy offer insight. An elevated baseline tryptase level plays a role in the safety of immunotherapy, but seemed to only be a risk for those on Vespid venom immunotherapy [65, 68]. An elevated tryptase level, not representing a mast cell disorder, did not seem to change the risk for systemic reaction for those on honeybee venom immunotherapy above the elevated risk these patients already carry. However, patients with a known mast cell disorder (e.g., mastocytosis) are an at-risk population entirely independent of the venom used in their immunotherapy and should be cautiously managed. A recent large, retrospective study using the same immunotherapy protocols and patient data collection for 10-plus years confirmed that honeybee is an independent risk factor for systemic reactions during venom immunotherapy [69]. In this study, it was also noted, however, that the patients on angiotensin-converting enzyme (ACE) inhibitors posed the strongest risk for systemic reaction. Interestingly, in another, albeit smaller, study evaluating risk factors for systemic reaction during immunotherapy specifically for honeybee, use of an

ACE inhibitor during immunotherapy, in a similar difference associated with elevated tryptase and honeybee, was not associated with higher risk [65]. In this study, a possible at-risk phenotype, those patients demonstrating a high basophil honeybee venom-specific CD63 sensitivity, was identified. This phenotype was a major risk factor for systemic reaction during the buildup phase for honeybee immunotherapy and confirmed suggestions of such from an earlier study [70]. In vitro BAT could be considered for honeybee-allergic patients to identify those at risk during immunotherapy, especially if it becomes more commercially available.

Monitoring for protection from honeybee immunotherapy would be helpful. It is currently not recommended, though, as associations with the presence of sIgG4 or disappearance of sIgE have not been reliably demonstrated [43]. Some recent attempts have been made looking at other methods. The BAT may be useful for this purpose as a decrease in basophil response was demonstrated in children on honeybee immunotherapy [71]. BAT may also be useful in assessing future failures after stopping venom immunotherapy [72].

When patients have failures (systemic reactions to stings) while on honeybee venom immunotherapy, it is reasonable to increase the dose in an effort to induce tolerance. Increasing the dose stepwise up to 400 μg per injection has been reported to gain protection, as shown by the disappearance of reactions or demonstration of very mild reactions following sting challenge [73]. Interestingly, for honeybee venom immunotherapy, doses as low as 50 μg have also been used and found to have failure rates similar to that of 100 μg doses [74, 75]. Despite these results, it is still generally accepted that the initial target maintenance dose of honeybee venom immunotherapy is 100 μg per injection [43].

The maintenance dose injection interval for honeybee immunotherapy is not unlike that of other flying Hymenoptera at 4–6 weeks [43]. Studies looking to extend the interval and possibly improve patient adherence while maintaining protection were initially performed on honeybee-allergic patients. It was found that after 12 months on maintenance immunotherapy, spacing the dose up to 8–12 weeks demonstrated comparable protection and is a reasonable option [76, 77]. However, extending the spacing to 6 months did not have similar results [78]. This interval recommendation is generally felt to be the same for all flying Hymenoptera [43]. In addition, a 3–5 year venom immunotherapy course is the standard of care for all flying Hymenoptera allergy. However, as honeybee venom allergy is so often associated with more immunotherapy failures and more severe reactions, life-long venom immunotherapy courses should be strongly considered in this population [43].

Although providing protection using subcutaneous venom immunotherapy is the standard of care, it is not the only method attempted to convey some protection. Passive immunotherapy using beekeeper serum gamma globulin was used in patients who continued to have sting reactions while on conventional immunotherapy and was found to offer protection and was felt to be safe [79]. The long-term viability (e.g., cost) or general application of such an intervention is certainly in question [80]. Also, sublingual venom immunotherapy was found to decrease large local reactions in honeybee-allergic patients [81]. Of course, the results of this study should not be extrapolated to include protection from systemic reactions [82]. Various other delivery

methods (virus-like particles, PLA_2-loaded microbubbles, poly[lactide-co-glycolide], etc.) and routes of administration (mucosal, intralymphatic, intradermal, etc.) have been explored in an effort to improve efficacy, safety, and patient adherence, but most remain experimental until more definitive studies can be accomplished.

Omalizumab administration appears to now be generally accepted as a method to allow those who fail venom immunotherapy to receive venom immunotherapy, as it has been found to decrease systemic reaction rates [83, 84]. Although omalizumab is often used in patients who fail honeybee venom immunotherapy, a recent study called into question its long-term use citing data that suggest only the safety is improved and not long-term protection, despite no longer reacting to sting challenges [85]. Regardless of this concern, it can still be strongly considered to facilitate those failing immunotherapy to achieve protection.

Unique Clinical Reactions to Honeybees

Honeybee Venom Sensitization/Reaction via Various Routes

Sensitization or reactions to honeybee venom can occur by various means and various bee products, despite most significant reactions are usually a result of a stinging incident. It was found that propolis, a substance described below, was ingested as a natural remedy for pharyngitis (commonly found in lozenges) and led to an anaphylactic reaction. The reaction was discovered to be a result of mucosal exposure to honeybee venom found in the propolis [86]. Royal jelly, also discussed in more detail below, often containing small amounts of venom, was ingested and caused a life-threatening systemic reaction [87]. One report demonstrated that a secondary exposure to honeybee venom from a previously used glass caused a generalized allergic reaction in a child [88]. Sensitization in up to 10% of all honeybee-allergic patients in Korea was found to be from Oriental medicine-specific acupuncture utilizing honeybee venom extract on the needles [89]. Ingestion of natural honey may also lead to sensitization [90] and anaphylaxis [91] since honey has been found to contain varying amounts of honeybee venom. Finally, intentional bee sting, apitherapy, is an alternative medical treatment of conditions such as rheumatism, pain, multiple sclerosis, cancer, and many others. Medical literature lacks support for its efficacy in treatment [92, 93], and there is inherent risk of systemic allergic reactions with intentional bee sting venom exposure.

Reactions from Honeybee-Related Products

In addition to unintentional honeybee stings causing allergic and toxic reactions, several other bee-related products have been identified as causing reactions. Exposures include bee pollen, propolis, honey, royal jelly, and beeswax.

Many of these products are found in various personal care items, nutritional supplements, eye contacts, foods, and beverages. These products are also often recommended for health maintenance and for treatment of numerous health-related conditions.

Bee pollen is marketed as a dietary nutritional supplement. After being ingested, allergic reactions occur due to the relative high concentrations of pollen, most often in those with a higher risk for respiratory pollen allergy [94], including children [95]. Both anaphylaxis [96, 97] and acute renal failure have been reported [98].

Propolis, known commonly as bee glue, is a resinous mixture bees collect from tree buds, sap flows, and other botanical sources. Reactions are primarily related to a product from the botanical source, but, in some, possibly a bee-secreted component. Propolis is found in natural cosmetics, toothpaste, dietary supplements, and many over-the-counter products. Among complementary and alternative medicine products, propolis is one of the most common components causing hypersensitive reactions [99]. Many reports have established propolis as causing allergic contact dermatitis (ACD) [100–103]. Due to its association with ACD, it has been studied and recommended for patch testing [103, 104]. A study of the North American Contact Dermatitis Group patch test results for 2007–2008 revealed propolis as the 11th most frequent positive test of 65 allergens tested [105]. Oral and hand ACD in musicians of wind and string instruments can be caused by exposure to propolis in those instruments' parts [106]. Less commonly, respiratory and acute renal failure has been reported [107] but also contact urticaria, rhinitis, and angioedema, usually from topical/mucosal application but also from ingestion [102, 108].

Honey is commonly consumed and occasionally administered topically. It has been found to contain nectar, honeydew, pollen, mold spores, bee body components, algae, and even venom. It rarely causes allergic reactions; however, due to pollen, bee secretions [109], and propolis [100, 110], reactions such as urticaria, facial itching, angioedema, and asthma have been documented [109, 111]. Though these reactions could be triggered by venom alone, it was found that some patients were able to tolerate ingestion of honey produced in a different geographical location, inferring that there may be other proteins in the honey itself that cause some of these reactions [109]. Also, anaphylaxis was reported in a case of honey containing Compositae pollen [112].

Royal jelly is composed of worker bee secretions as a source of food for bee larvae and adult queens. It is composed of 67% water, 12.5% crude proteins including small amounts of amino acids, 11% simple sugars, 5% fatty acids, and trace amounts of minerals, enzymes, antibacterial components, and vitamins. It is marketed as a dietary supplement but is sometimes used topically as well. Reactions, such as contact dermatitis, oral allergy syndrome, and asthma exacerbations have been well documented, as well as anaphylaxis and death [87, 112–117]. Higher risk for reaction exists for those with other allergies [117], and there is a report of cross-reactivity with dust mite [118].

Beeswax is a product made from the honeycomb. It is used in cosmetics, as a fragrance for soaps and perfumes, in foods and beverages, and as a medicine. It appears to be a relatively safe product, but cheilitis has been reported [119].

Beekeepers

Beekeepers, and others with a significant exposure to bees and beehives such as the beekeeper's family and honeybee researchers, represent a high-risk group for bee-related allergy due to their increased exposure. Risk for a honeybee sting causing a systemic reaction in beekeepers ranges from 4.4% to 38% [120–122], several times that of the general population. Many risk factors exist that are unique to beekeepers. The average annual bee stings among beekeepers is 57 [122]. Systemic reactions occurred in 45% of beekeepers with less than 25 stings per year [120]. Conversely, greater than 200 stings per year is protective [120]. Some protection occurs with greater than 50 stings per year [120]. Also, risk for systemic reaction is higher with first stings of the bee season, usually spring, with upper respiratory symptoms while managing hives, with first years of beekeeping, and if the beekeeper has other atopic diseases [123]. However, another study found increased systemic reactions after 2 years of beekeeping (and no prior systemic reaction) and if the beekeeper had family members who were already bee allergic, but no increased risk associated if the beekeeper had other allergic disease [121].

Beekeepers, compared to the general population, had lower levels of bee venom-specific IgE, lower skin sensitivity to the venom, and higher bee venom-specific IgG. However, there was no difference in severity of systemic reactions [120, 124, 125]. Also, among non-anaphylactic reactions to bee-related products, propolis was found to be a significant cause of allergic contact dermatitis in beekeepers due to their exposure managing hives [101, 126, 127].

In Southern states of the United States where Africanized bees (AHB) are established, there is an increased risk to beekeepers due to the more aggressive AHB taking over domestic beekeeper hives and increased probability of stings. There may be more or less risk in some beekeepers due to the increased number of stings per year, as noted above. Beekeepers also have a higher risk for multiple and massive stinging envenomation once AHB are present in their domestic beehive. Another feature which may make a difference in risk between Southern and Northern states is the shorter Southern winter leading to a shorter bee sting-free timeframe, resulting in less risk with the season's first bee stings for those in the Southern states.

Finally, a study identified increased risk as beekeepers often do not access the healthcare system as recommended for bee venom allergy. Following beekeeper systemic reactions, allergy specialists were seen by 16.6%, and epinephrine autoinjectors were acquired by only 18% [121]. Therefore, beekeepers are less educated about future reaction risk, epinephrine availability, and bee venom

immunotherapy. Avoidance is difficult as beekeepers continue their beekeeping activity and exposure despite systemic reactions; however, some will use more protection [121].

Unusual Reactions to Honeybee Stings

Rare and unusual reactions have been reported, often associated with unknown mechanisms of action. One to multiple bee stings causing these reactions are listed here, with the exception of those in the AHB section. Not unique to honeybees, Robert Reisman has documented unusual reactions to a variety of stinging insects, particularly involving neurological, renal, cardiovascular, pulmonary, hematologic, and ocular systems [128].

Reports of unusual reactions to honeybee stings including acute myocardial infarction caused by one and multiple bee stings, Kounis Syndrome, have been documented [129, 130]. Other reactions include acute disseminated encephalomyelitis [131], encephalopathy [132], peripheral neuritis [133], nephrotic syndrome [134], nephrosis [135], pulmonary hemorrhage [136], thrombocytopenic purpura [137], and hemorrhagic pancreatitis [138].

Africanized Honeybees

Introduction

Africanized honeybees, *Apis mellifera scutellata*, geographic range expanded into the United States (US) in 1990, and this remarkable expansion continues in the USA today. This has altered our approach to feral and "managed" bee colonies. Once AHB become established in an area, domestic feral bees' dominant traits change almost 100% to those of AHB. The aggressive response by AHB to actual or perceived hive disturbance is the cause of massive numbers, thousands of bees, to shift into "attack mode" stinging any human or animal in the vicinity. This is the reason AHB have been commonly referred to as "killer bees." However, it is the number of stings and therefore total volume of venom injected, not the volume or components of venom in individual bee stings that has resulted in a unique clinical condition of venom toxicity. A wide range of treatment from mild supportive care and observation to intensive life support measures is dictated by clinical presentation and degree of envenomation. A very important factor that can often predict clinical presentation is the number of stings. Mortality is fortunately rare but significant, especially in high-risk situations. Finally, in areas where AHB are present, people's views changed along with their approach to foraging honeybees and their hives as well as public safety concerns remarkably altering first responder protocols.

Background

Honeybees from Africa were brought to Brazil for research in an attempt to find a more suitable honeybee for their tropical climate. Domestic, or European honeybees (EHB), *Apis mellifera*, multiple subspecies, had some difficulty in the Brazilian environment. In 1956, multiple African honeybee colonies were released or "escaped" the research facility. Interbreeding with feral domestic honeybees resulted in the hybrid AHB, which maintained most of the dominant African honeybee traits and survivability.

Range expansion was rapid, especially northward in South America, Central America, and Mexico. In 1990, 34 years later, the first AHB colony by range expansion in the USA was identified in Hidalgo, Texas, the southernmost area of Texas. From there, Texas range expansion of AHB, as well as all US-Mexico border states, and into states north and east of border states over the years was significant. As noted in the maps of AHB habitation (2014) and habitat suitability, further range expansion is predicted but is approaching expected geographic limitation due to temperate climate. However, any area of the USA could encounter AHB at least temporarily due to transport by rail, vehicles, sea vessels, and managed bee colony transport.

See maps—Geographic Spread—Fig. 10.4 and Habitat Suitability—Fig. 10.5.

Although AHB are slightly smaller than EHB, there is no ability to visually distinguish between the two; however, mitochondrial DNA analysis is an accurate lab method to distinguish AHB from EHB. [139].

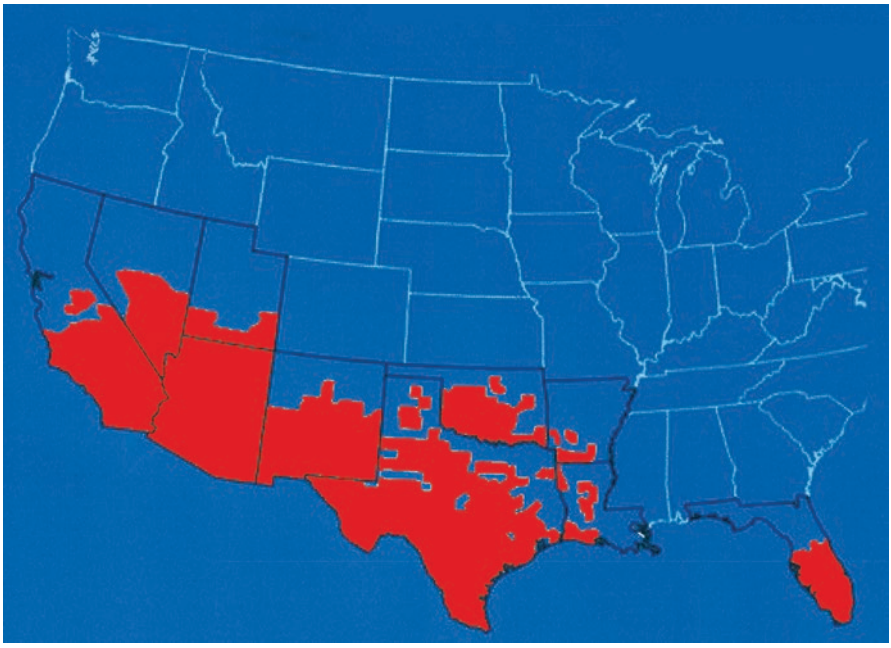


Fig. 10.4 Recent habitat of Africanized honeybees in the United States

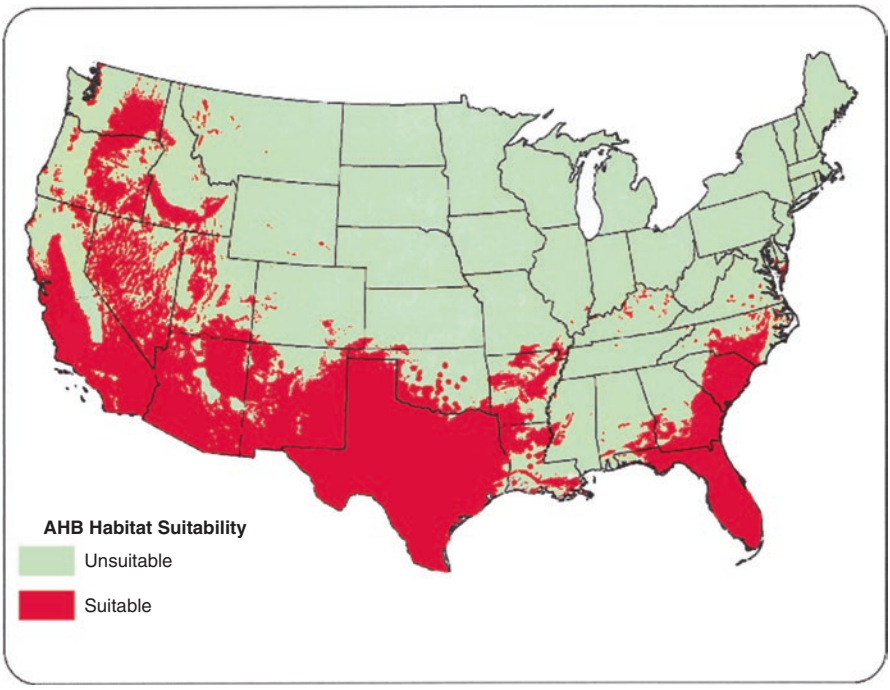


Fig. 10.5 Africanized honeybee habitat suitability in the United States

Behavior-Venom

AHB establish their hives in locations not traditional for feral EHB hives. It is common to encounter AHB hives in fence and wall holes, dry cracks in the ground, old tires, and under mobile homes, to name a few. AHB swarm more frequently, establish more colonies per area, and are more disease resistant than EHB. These characteristics exemplify why this honeybee subspecies has survival sustainability, range expansion capabilities, and environmental impact. Unfortunately, it also explains why there are more accidental and intentional AHB-human encounters and resultant issues.

One study demonstrated the more aggressive behavior of AHB in comparison to EHB [140]. In the study, the beehive was agitated, then sprayed with a synthetic alarm pheromone similar to that released by bees when their hive is threatened. A red moving "target" was placed in front of the hive entrance, which received the stings. The response was more rapid, and the "target" received eight times more stings from AHB than EHB Fig. 10.6.

Venom composition is nearly identical between EHB and AHB [141]. There is a higher amount of PLA_2, a lower percent of mellitin [142], and a lower volume of venom per venom sac (AHB 93.5 μg vs. EHB 146.6 μg dry weight) [143] of AHB than EHB. Proving this point, one study demonstrated similar toxic and neutralization of toxic effects between AHB and EHB venom in mice [143].



Fig. 10.6 Massive Africanized honeybee response

Allergic Reactions vs. Toxic Reactions and Venom Components

Clinicians as well as the general public are aware of a potential immediate IgE hypersensitivity reaction that occurs in a small percent (1–4%) of the general population. This reaction is discussed in other chapters of this book and will not be addressed here, with a couple of caveats. Toxic reactions occur with virtually all stings with envenomation usually the well-recognized, "normal" small and occasionally large local reaction. However, multiple stings result in clinical presentations beyond local skin reactions, and higher numbers of stings with massive envenomation will predictably result in systemic toxic reactions. These toxic reactions can occur with any honeybee subspecies but certainly have become a higher health risk with AHB range expansion.

Three components of bee venom cause most of the toxicity contrasting a different perspective from allergy clinically. These include melittin, PLA_2, and hyaluronidase [144–146]. In addition, apamin; mast cell degranulating peptide; vasoactive components including histamine, dopamine, and norepinephrine; and other venom components add to toxicity. Melittin and PLA_2, as noted previously, cause disruption

of the cell membrane, with associated arachidonic acid release and leukotriene generation. This results in cell membrane breakdown, damage to endothelium, and activation of proinflammatory cytokines (IL-1, IL-6, IL-8, and TNF) [146–148]. Hyaluronidase is a spreading factor causing vasodilation and fluid extravasation, disseminating venom components, thus inferring more widespread toxic venom exposure [148, 149]. Melittin also leads to vascular cellular component toxicity and hemolysis [149, 150]. Apamin is a neurotoxin, causing central nervous system excitation [147]. Collectively, directly and indirectly, these toxic components can affect all tissues, but there is more clinical involvement of cutaneous, muscle, renal, hepatic, cardiovascular, respiratory, and hematologic tissues. As an example of comparative toxicity, the systemic toxicity of a middle-sized rattlesnake's venom (entire venom compartment) is roughly equivalent to the toxicity of 500 honeybees' venom (J.O. Schmidt, PhD., oral communication).

The clinical effects of these toxic reactions are mostly predictable on the basis of the effects of venom components and the collective volume of venom exposure, calculated by the "number of stings" and patient's weight. However, less predictable groups, those with higher risk of toxic reactions and complications even with lower numbers of stings/venom exposure, include young children, underweight individuals, the elderly, and those with significant underlying medical conditions [151, 152].

Sting Site Considerations

The site of a disturbed AHB hive resulting in the response of thousands of bees in "sting mode" flying rapidly and chaotically in a crisscrossing pattern over a somewhat dome shaped area of 50–100 yards in diameter seeking a sting "target" is extraordinary and dangerous. Once a human and/or animal "target" is stung, a marker pheromone in bee's venom signals other bees to the "target," and multiple or massive numbers of stings/envenomation immediately occur. AHB pursue victims up to a quarter of a mile [153], occasionally longer, from the sting site although less bees pursue with distance. The most important action for the stinging victim is to seek distance from the site or preferably into any enclosure such as a vehicle or dwelling as fast as possible. Once in an enclosure, the sting number is limited to those accompanying the victim, and many of those bees have already stung the victim. Any bystanders should call/encourage the victim to move away from the area or into a vehicle or other enclosure. This is a dangerous site and, in urban areas, a public health safety emergency. Emergency response should be activated. Stingers and their attached venom sac should be removed as rapidly as possible with a blunt item such as a credit card and butter or plastic knife to remove the stinger at the skin–stinger interface being careful not to "pinch" the stinger and inject more venom [154]. There are many reports of victims covered with bees, flailing of arms and other random motions in an attempt to avoid the attack, screaming, spraying water trying to remove bees, and other unhelpful actions while the sting count mounts. If the victim does not take evasive action to limit stings within a minute or

shortly thereafter, a psychological aura of indifference occurs with the victim aimlessly wandering, standing, or sitting. If rescue is not imminent, collapse follows. Post sting communication with victims describes a shock-like state and a "numbness" sensation to any discomfort or personal safety concern. Several articles have documented remarkable reactions [151, 155–161].

In the prior scenario, if the victim is not actively moving to safety and first responders, primarily firefighters in their protective "turn out" gear, are not on the scene for rescue, a rescue may be attempted with partial protection by using a blanket, tarp, large towel, shirt, or similar item "hooded" over the rescuer's head [154]. Rush to the victim and pull, drag, or carry the victim to a safe location. The rescuer(s) will receive multiple stings and are sometimes overwhelmed before successful rescue, resulting in an aborted rescue attempt. The author was involved in the case in which the collapsed victim's husband attempted three rescues unsuccessfully before first responders arrived. He received 340 stings and required hospitalization with a moderately high toxic reaction.

Clinical Presentation, Evaluation, and Treatment of Multiple and Massive Envenomation

Upon the arrival of the honeybee-sting patient to a healthcare facility, outpatient clinic, urgent care, or emergency department, the patient's general appearance and accompanying persons or EMT's information can help to immediately determine the patient's status. History of allergic reactions to stings in the past; quick assessment to determine few, many, or massive sting exposure; traumatic injury involved; underlying medical conditions; and medications are all important. If a brief-focused history and vital signs/physical exam are stable, a more accurate sting count can then be determined. Removal of stingers and attached venom sac is effective at limiting envenomation with the pulsating stinger/sac apparatus as soon as the patient is safely away from the sting site. However, in the healthcare facility, it is not helpful as all venom is injected within 1 min of the sting [18, 149]. Counting the number of stings is a daunting task when the sting count is in the 100-plus range, but the higher the number of stings, the more important the count. One recommended approach is to count half of the body areas stung and multiply by two. Body stings are usually relatively symmetrical and can be verified with a brief visual examination. The head and neck area receive a high percent of stings followed by the upper extremities, since the victims are usually wearing a short-sleeved shirt. Skin-exposed areas usually receive a high majority of stings. Count the right or left half of the head and neck area, one upper extremity, etc., and multiply by two. Again, sting numbers are very useful to assess level of venom exposure and toxicity.

Picture of Massively Stung Patient—Fig. 10.7.

Common patient symptoms at presentation of a significant envenomation include various cutaneous symptoms, anxiety, dizziness, headache, and weakness. Above 50 stings, common symptoms additionally include pain, nausea, vomiting, and



Fig. 10.7 Extreme level of stings by Africanized honeybee

diarrhea [145, 149, 151, 162–166]. At the highest levels of envenomation, additional symptoms include remarkable malaise, pain, shortness of breath, and altered levels of consciousness. Clinical findings of cutaneous lesions consistent with bee stings reveal many with stingers and attached "dry" venom sac. Vital signs often are stable with the exception of tachycardia and hypertension. At the highest levels of envenomation, but may occur with lower levels, an unstable patient is treated with established intensive and life support medical protocols.

Little information is known regarding the combined condition of IgE hypersensitivity and multiple or massive envenomation. Again, clinical presentation dictates treatment. The author has seen a case of a patient with bee venom IgE hypersensitivity, anaphylaxis, on bee venom immunotherapy maintenance dose (100 μg monthly injection) who received 44 stings, and the only symptom was mild malaise for 2–3 days. No epinephrine was administered, medical evaluation or other medical treatment.

Laboratory evaluation usually consists of complete blood count (CBC), creatinine phosphokinase (CPK), cardiac enzymes, comprehensive metabolic panel, urinalysis, coagulation tests, and other tests as indicated, such as tryptase level, blood gases, and chest x-ray. Abnormal labs, which commonly parallel the degree of venom toxicity and clinical expression, include CPK (both skeletal and cardiac muscle factions), liver and kidney function tests, and blood cellular components. CPK is a reliable marker for the presence and intensity of muscle injury and usually peaks on day 3–4 [155, 167–170]. Urine testing for myoglobinuria and hemoglobinuria as well as other abnormalities should be monitored. Often lab tests are normal or only mildly abnormal at admission, with the exception of commonly elevated neutrophilic leukocytes. Therefore, daily or more frequent assessment of CPK, liver and kidney function tests, CBC, and urine testing is recommended along with urinary output monitoring and any other evaluation, as clinically indicated.

During the patient's hospital course, multiple complications may occur. Rhabdomyolysis and acute renal failure (ARF) is due to pigment nephropathy associated with direct toxic effects of myoglobinuria and hemoglobinuria. Also, ARF may be associated with a toxic ischemic mechanism with hypovolemia and/or hypotension. Finally, ARF may cause acute tubular necrosis by direct venom toxicity [147, 151, 155, 161, 163, 164, 167–178]. Cardiac dysrhythmias occur. Interpretation of elevated cardiac enzymes is difficult as venom toxicity causes general cardiac muscle breakdown, but acute myocardial infarction (MI) may occur. Kounis syndrome [129] has been defined as an association of a hypersensitive reaction to multiple bee stings and acute MI. Disseminated intravascular coagulation and respiratory distress and failure also may occur [143, 144, 179].

Treatment ranges from observation to life support measures. Treatment is dictated by clinical presentation, lab results, clinical course, and degree of envenomation. The initial presentation may be deceptively mild, especially when the exposure is to higher levels of envenomation. Clinical and laboratory status often abruptly worsen hours to days after envenomation. Further deterioration of clinical status may then occur [145, 155, 171, 180]. Fluid replacement is very important in the vast majority of patients. In the clinically stable patient, those with venom exposure of moderate levels, one to four stings/kg, and higher, probably the most important initial treatment is an immediate intravenous (IV) fluid bolus. Patients are usually hypovolemic due to multiple factors. Victims are usually working outdoors, often for hours, in warm to hot weather without significant fluid intake. Often victims have vomited multiple times and sometimes have diarrhea. Venom components also cause extravasation enhancing hypovolemia and fluid sequestration in damaged skeletal muscle and other tissues. The hypovolemic state intensifies venom's toxic effects. While pushing IV fluids, urinary output must be monitored. Although no well-controlled studies have proven effective treatment guidelines, additional common treatments include skin care (after sting count), antihistamines, corticosteroids, and pain and antiemetic medication. Alkalization of urine can help protect renal function. Oxygen, vasopressors, mechanical ventilation, and other measures are occasionally indicated, and later in the clinical course, hemodialysis may be indicated [143, 146, 156, 181]. Unlike IgE hypersensitivity reactions to bee venom, epinephrine is not routinely recommended and can worsen the toxic reaction. Only administer epinephrine if clinically indicated.

Plasmapheresis, or exchange transfusion, has the ability to remove venom and its toxic effects, cellular debris, and inflammatory mediators from the blood's circulation [156, 171, 182]. Three clinical presentations should be considered for these invasive but potentially lifesaving procedures. First, a patient with a severe initial clinical presentation with usually very high, 9–19 stings/kg or extreme exposure >19 stings/kg, but occasionally lower exposure, especially in the elderly or those with significant underlying medical conditions should be considered an immediate indication. A second indication is a patient without a severe initial clinical presentation but has a sting count near or >19 stings/kg. Over the following hours to days, clinical status and laboratory values reflective of toxicity will predict a poor prognosis, with organ failure, and may be life threatening. For these first two situations,

there is a brief therapeutic window of opportunity for consideration of this procedure. The final indication is if the initial clinical presentation and sting count are not reflective of the prior two scenarios but clinical deterioration occurs over the following hours to days and significant lab abnormalities consistent with severe toxicity occurs. In addition, acute renal failure may necessitate dialysis. Fortunately, most of these cases have required dialysis temporarily [146, 156, 167].

Since healthcare providers see patients of various levels of clinical presentation and venom exposure, a guide is presented (Table 10.2) for evaluation and treatment. Examples of various patient weights and sting count (stings/kg weight) are presented (Table 10.3) and classify the level of venom exposure.

Table 10.2 GUIDE FOR MULTIPLE AND MASSIVE NUMBERS OF HONEYBEE STINGS. Proposed guide for the degree of venom exposure, predicted clinical expression, evaluation, and management (not including immediate hypersensitivity IgE reactions, > age 65 years, and those with significant underlying medical conditions). **Rapid sting count assessment mandatory** (see text for sting count methods).

Mild venom exposure—multiple stings <1 sting/kg wt.—usually cutaneous symptoms only. Topical treatment for hygiene and discomfort. Optional evaluation in a healthcare facility. Near the one-sting limit or any additional symptoms recommend evaluation and treatment in a healthcare facility
Moderate venom exposure—one to four stings/kg wt.—expect clinical symptoms, in addition to cutaneous symptoms, pain, anxiety, most common, followed not uncommonly by nausea and vomiting, then abdominal pain, and diarrhea. Cardiovascular findings, tachycardia, and hypertension are common. Less common are hypotension and renal function compromise. This level rarely results in an unstable presentation requiring intensive management. Evaluation in a healthcare facility and 24-h observation or admission is recommended. Mild lab (see text for recommended tests) abnormalities which may worsen with delayed toxic reactions over the following 48 h. Initial IV fluid bolus is important in most patients. Then outpatient follow-up is recommended
High venom exposure—four to nine stings/kg wt.—moderate to severe clinical presentation (symptoms, but more expressed, as listed with moderate venom exposure prior) but may be milder clinical presentation at lower range of exposure. Multiple organ systems affected, GI tract, cardiovascular, hematologic, renal, hepatic, and pulmonary. Pain is often significant. Multiple lab (see text for recommended tests) abnormalities typically worsen over 48–96 h. IV fluid bolus is the most important initial treatment in stable patient. Admission and often intensive management and, occasionally, life support measures, as indicated
Very high venom exposure—9–19 stings/kg wt.—increasing severity (see high venom exposure) and not uncommon to present with altered mentation from combative to unconscious. Multiple organs affected. Recommend intensive care management with constant monitoring. Possibly invasive measures such as plasmapheresis or exchange transfusion started within hours if initial clinical presentation is very severe or a deteriorating clinical course. Remarkable lab abnormalities. Later in the clinical course, dialysis due to acute renal failure is occasionally indicated
Extreme-lethal venom exposure—>19 stings/kg wt. (LD 50% estimate, but not strongly established, at 19 stings/kg) [149, 180, 183]—remarkable pain and/or altered level of consciousness. Rapidly deteriorating clinical course with multiple organ failure. Intensive and life support management. Plasmapheresis or exchange transfusion should be considered immediately if available

Table 10.3 Weight and sting count/venom exposure level

	Mild	Moderate	High	Very high	Extreme
Weight	<1 sting/kg	1–4 stings/kg	4–9 stings/kg	9–19 stings/kg	>19 stings/kg
20 kg	<20	20–80	80–180	180–380	>380
50 kg	<50	50–200	200–450	450–950	>950
80 kg	<80	80–320	320–720	720–1520	>1520
100 kg	<100	100–400	400–900	900–1900	>1900

Mortality

Deaths due to AHB stings are rare. There are no studies in the USA that accurately report mortality, but reports of multiple deaths, up to ten per year, are estimated. Most deaths are associated with the elderly and those with comorbid conditions. Some occupations and activities place individuals at higher risk for mortality such as operating machinery, especially alone clearing land in rural areas where help and access to health care is significantly delayed.

There have been fatal reactions with low numbers of stings. The author was involved with a fatal case of an 82-year-old male with 50 stings and a history of cardiopulmonary disease. Autopsy revealed massive pulmonary edema and no serologic evidence of bee venom IgE. Also documented is survival of healthy adults with greater than 2000 stings [156, 157].

A study of mortality in Mexico spanning the years of 1988–2009 revealed an average of 21 deaths (documented) per year [184]. Another study documented at least 70 deaths in the initial years of AHB, 1978–1981, in Venezuela, at that time a country of 14 million [185].

Follow-Up of Those with Multiple and Massive Envenomation

When a patient is discharged from a healthcare facility, follow-up evaluation and laboratory is recommended. In mild venom exposure cases, follow-up is mainly to evaluate possible delayed, toxic reactions. After hospital discharge, it is recommended to follow up the patient's clinical status and any prior abnormal lab until values normalize. CBC, CPK, renal and liver function tests, and urinalysis are recommended as well as any other lab, as per clinical course or comorbidities. Of interest, in a small study of 13 patients, over 50% patients with >50 stings had positive skin tests and/or RAST to bee venom [151]. Significance of this finding is unknown. Additionally, since it is known multiple individual stings are an increased risk for allergic sensitization, it may be recommended that the patient be evaluated in follow up for allergic sensitization and consider as a candidate for venom immunotherapy. The author has seen patients who have symptoms over many weeks to months following recovery. These symptoms include lethargy, poor appetite, paresthesia, and sun sensitivity, which usually slowly improve.

Avoidance

Several measures can prevent the majority of situations resulting in multiple and massive envenomation. Never intentionally disturb a hive unless highly trained and properly equipped. Before operating machinery in residential and commercial landscape activities or rural land or roadway area clearing, inspect the area. AHB are conspicuously active in the immediate vicinity of their hive. Do not disturb or operate machinery in that situation unless protected in special "bee suits," enclosed tractor cab, or similar. Even if one takes these precautions, if a hive is disturbed and become aggressive, there is danger to others in the area.

Potential Direct Treatment of Massive Envenomation and/or Severe Toxic Reactions

Anti-bee venom treatment has the potential to neutralize honeybee's toxic effect, analogous to anti-snake venom's well-established efficacy. Studies in animal models have shown highly effective results when beekeeper's hyperimmune globulin is injected into mice resulting in neutralizing bee venom's toxic effects [143]. Unfortunately, no such product is currently available, but if or when available would be expected to be the most effective direct treatment for patients with massive envenomation and any patient with a remarkable clinical toxic reaction, even in those with lesser number of stings.

Conclusion

Honeybees have unique characteristics from other stinging insects resulting in a variety of interesting and challenging issues. Their usual docile nature is necessary for survival of the species. Due to the barb structure of the stinger, the stinging event causes the stinging apparatus to remain in the skin. This results in evisceration and the bee's demise. Venom components, many unique to honeybees, present a variety of diagnostic and therapeutic challenges. Due to commercial benefits of honeybees, bee-related products are utilized by various routes of exposure and resultant sensitization reactions. Beekeepers, their families, and researchers have health risks due to their increased exposure. Honeybee colonies have huge numbers, many thousands, of bees per colony thus increasing health risk when a colony is disturbed, intentionally or not. Occasionally, especially where AHB are established, multiple and massive sting events occur and are a true medical emergency for the individual's stung, an unstable area, and public health emergency in populated areas. For the first time, an abbreviated guide is proposed which outlines levels of honeybee venom exposure, clinical expression, evaluation, and treatment.

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Chapter 11
The Imported Fire Ant: The US Experience

Mike Tankersley and Daniel Steigelman

Introduction

Ravaging the southeastern USA for most of the last century, the imported fire ant (IFA) has a distribution among the continents of Asia, Australia, and North and South America [1]. A native species in Argentina, the red IFA, *Solenopsis invicta,* was introduced in the ballast of ships docking in the port of Mobile, Alabama, during the 1930s and now is the prevalent species in the USA [2]. The black IFA, *S. richteri*, is confined primarily to the northeastern part of Mississippi. Using genetic markers (allozymes, microsatellites, and mtDNA sequence) to compare IFA found in Mississippi and Louisiana with native fire ant populations in South America, the original source of fire ants is most likely from Argentina in the Mesopotamia region close to Corrientes and Formosa. Previously assumed to be introduced from Brazil, no ants from Brazil shared markers with *S. invicta* ants sampled from the USA [3]. The IFA has spread to affect over 300 million acres in the USA, including 14 states and the Commonwealth of Puerto Rico. In the USA, the IFA have inflicted over five billion dollars in annual economic damage [4]. Beyond the borders of North and South America, the IFA also affects portions of Australia, China, New Zealand, and Taiwan [1].

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M. Tankersley, M.D.
The University of Tennessee Health Science Center, Memphis, TN, USA
e-mail: mtanker6@uthsc.edu; mandjtank3@yahoo.com

D. Steigelman, M.D. (✉)
Department of Allergy and Immunology, San Antonio Uniformed Services Health Education Consortium, Wilford Hall Ambulatory Surgical Center, San Antonio, TX, USA
e-mail: daniel.steigelman@us.af.mil; daniel.a.steigelman.mil@mail.mil

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Sting Attack Rates

The IFA have a reputation for being aggressive with the disturbance of an IFA mound immediately resulting in a swarm of ants from the mound that respond by stinging an unsuspecting animal or human invading their space [5]. The pathognomic manifestation of a sting is the formation of a sterile 1–2 mm pseudopustule resulting from the venom piperidine alkaloids which provide the insecticidal, bactericidal, and fungicidal activity of the venom [6]. Native fire ants, including *S. geminata*, *S. aureus*, and *S. xyloni*, share venoms cross-reactive with *S. invicta*. Sol i 1 and 3 antigens are more conserved than Sol i 2, which may have species-specific epitopes. *S. geminata* shares Sol i 4 with *S. invicta* [7].

High sting attack rates have been reported in those visiting or residing in IFA-endemic areas. A prospective study of 107 medical students from non-endemic IFA areas visiting San Antonio, Texas, for 3 weeks during the summer reported a sting incidence of 51% [8]. None of these students reported generalized cutaneous or systemic reactions (SR). Seven of the students who were stung (12.7%) developed IFA skin test reactivity. Serologic testing (RAST) was positive in only one of the students. In a second report on sting attack rates, a survey of 182 children living in an IFA-endemic area reported 38.6% were stung by IFA over the previous month and 23.9% had more than six stings [9]. Of all insect stings reported, IFA accounted for 92.1% with flying Hymenoptera accounting for only 7.9%. Pediatric patients were at greater risk for IFA stings in a rural environment than suburban or city environments. In this same study, random screening of discarded serum in IFA-endemic areas demonstrated IFA-specific IgE >0.35 kIU/L detectable in 38.3% of children, varying with age from 35.7% in 2–5 years old to 57.5% in those aged 11–20. In contrast, screening of adult blood in an IFA-endemic area found IFA-specific IgE ≥0.35 kIU/L detectable in only 17% [10]. Yellow jacket venom (YJV)-specific IgE was detectable in random serum screening of 7.6% of pediatric patients and 10% of adults. This compares with random adult screening of adult blood in non-endemic IFA areas where YJV-specific IgE was found more commonly than IFA-specific IgE (6.5% and 2%, respectively). In a separate study in an IFA-endemic region, blood samples from 145 unselected children were screened for sensitization to IFA, YJV, and peanut. Seventy (48%) were sensitized to IFA, while 11 (7.6%, $p < 0.0001$) were sensitized to YJV and 16 (11% $p < 0.0001$) to peanut [11]. IFA-specific IgE values ranged from 0.35 to 68.8 kAU/L with a geometric mean of 3.07 kAU/L. Ten percent of IFA-sensitized children were also sensitized to YJV.

A Common Problem

Patients with a history of systemic sting reactions to IFA and other Hymenoptera are frequently referred to practicing allergists in IFA-endemic areas. Sensitization to IFA is frequently more common than to any other single Hymenoptera and has

been found nearly equal to the prevalence of sensitization to all other Hymenoptera. A 5-year retrospective medical record review of 769 records from two military medical facilities in IFA-endemic areas (San Antonio, Texas, and Charleston, South Carolina) and coded as relating to an insect sting were reviewed [12]. Of these 769 charts, 120 (15.6%) had anaphylaxis and, of these, 46 (38.3%) were due to IFA, 59 (49.2%) to flying Hymenoptera, 5 (4.2%) to scorpion, and 10 (8.3%) unspecified. Forty-seven of the 120 patients (39.2%) were referred to an allergist and, of 34 who kept their referral appointments, 25 of 28 (89%) who were skin tested demonstrated sensitization and immunotherapy was recommended. In a separate study of 703 patients in an IFA-endemic area referred over 10 years to a military allergy clinic for venom allergy, 515 (73%) had a history of reaction consistent with anaphylaxis, and 315 (45%) had a positive history and skin testing [13]. The leading cause of Hymenoptera hypersensitivity was IFA (42% of patients), followed by wasp (29%), then hornet (16%), yellow jacket (15%), and finally honey bee (12%). In this study, the youngest patient offered immunotherapy was 18 months of age and was allergic to IFA. A retrospective survey of practicing allergists in the Dallas-Fort Worth area to which 38 provided written responses (74.5%) estimated 3.8% of their patients were on immunotherapy for IFA, and an estimated 1.1–2.1% of new patients seen in the past 12 months were referred for an IFA SR [14].

Reactions

An IFA sting reaction typically results in a characteristic sterile pseudopustule but may also include a large local reaction (LLR), may be solely confined to generalized urticaria, or may manifest with systemic symptoms of anaphylaxis. LLRs are seen in 17–56% of patients [6]. A study of pediatric patients with LLRs or cutaneous reactions reported no progress to SR with future stings [15]. In this cohort of 31 children ages 16 years and younger evaluated for local or cutaneous reaction to IFA, only 20 had a future sting with none of these children developing an SR over a mean follow-up period of 4.9 years. Utilizing a survey which was completed by 2022 physicians who had treated 20,755 patients for IFA stings, it was estimated that 413 (2%) had been treated for life-threatening anaphylaxis [16]. In a separate survey, 2506 physicians reported 84 deaths from IFA stings, most of which resulted from less than five stings [17]. Ten indoor IFA stings previously unreported in the medical literature were found as a part of a review of newspaper articles over a 15-year period [18]. When reviewed with ten previously reported indoor IFA stings, 14 of these 20 occurred in healthcare facilities (11 stung in nursing homes or assisted living or residential care facilities, 3 in hospitals, and 5 in private homes) and over 50% involved patients immobilized by disease and/or age (e.g., a 26-month-old boy with hydrocephalus and developmental delay, an 83-year-old man with stroke and dementia, a 91-year-old woman with a fractured hip). Finally, a total of six stings resulted in death within 1 week in those ages 3 months to 90 years.

Individual case reports demonstrate IFA morbidity and mortality in vulnerable populations. Discovered during a routine check in a mobility-impaired nursing home, a 67-year-old patient with dementia had numerous IFA on the their arm, chest, neck, stomach, and back, leaving multiple wheal and flare reactions [19]. A trail of IFA from the patient's bed to the floor was found. The patient's chronic congestive heart failure worsened and the patient died 5 days after stings. In another nursing facility, a 60-year-old patient with hemiparesis following cerebrovascular accident fed by percutaneous enterostomy tube was found covered with IFA during a routine check. While the patient did not have signs of anaphylaxis, he developed LLRs and then pneumonia and congestive heart failure. IFA were found in a trail from the floorboards and over the furniture to his bed. In both of these cases, pest control services had been to each facility within 4 days prior to the attacks and did not find evidence of IFA infestation. Native fire ant species' stings may also be life-threatening. *S. xyloni* is found in the southwestern USA and Mexico. A 3-month-old infant in a daycare setting was found apneic and covered with ants after an episode of crying. Postmortem examination was remarkable for the absence of pustules typical of *S. invicta* and *S. richteri*, serum was remarkable for elevated tryptase (23.9 ng/mL), and radioallergosorbent test (RAST) demonstrated 1.4% specific IgE antibody binding to *S. richteri* venom but negative to *S. invicta* venom. Identified by an entomologist, *S. xyloni* ants were recovered from the scene [20].

Evaluation and Treatment

In the evaluation of a patient with a history consistent with IFA hypersensitivity, skin testing should be performed with IFA whole-body extract (WBE). Skin testing should be initiated via prick with a concentration of 1:1000 w/v, and, if negative, intradermal testing should then be undertaken with serial concentrations starting at 1:1,000,000 w/v to a maximum concentration of 1:1000 w/v. Sensitization may also be determined with serum-specific IgE to IFA. As with all forms of anaphylaxis, epinephrine is the treatment of choice for IFA anaphylaxis (0.01 mg/kg for children to a maximum of 0.3 mg and 0.3–0.5 mg for adults) [21]. Administration via the intramuscular route in the anterolateral thigh is recommended. Physicians should prescribe and instruct IFA-allergic patients to carry or have available an autoinjectable epinephrine device for use in outdoor and indoor environments. For those patients with IFA hypersensitivity, initiation with IFA WBE subcutaneous immunotherapy is recommended. A targeted maintenance dose of 0.5 mL of a 1:100 w/v extract (Table 11.1) should be utilized. For those patients experiencing field sting reactions while on maintenance immunotherapy doses who are considered treatment failures, consideration should be given to using a higher maintenance dose. As children living in endemic IFA areas are at a significant exposure risk, those who experience cutaneous reactions should be considered for IFA immunotherapy [21]. A study of IFA WBE individually mixed with aeroallergen extracts demonstrated stability of IFA WBE by SDS-PAGE and after 6 months of incubation ELISA, radial

Table 11.1 Example of a conventional IFA WBE immunotherapy dosing schedule [21]

Dose	Concentration (wt/vol)	Volume (mL)
1	1:100,000	0.05
2	1:100,000	0.15
3	1:100,000	0.25
4	1:100,000	0.50
5	1:10,000	0.05
6	1:10,000	0.10
7	1:10,000	0.20
8	1:10,000	0.30
9	1:10,000	0.40
10	1:10,000	0.50
11	1:1000	0.05
12	1:1000	0.10
13	1:1000	0.20
14	1:1000	0.30
15	1:1000	0.40
16	1:1000	0.50
17	1:100	0.05
18	1:100	0.07
19	1:100	0.10
20	1:100	0.15
21	1:100	0.20
22	1:100	0.25
23	1:100	0.40
24	1:100	0.50

Observation time 30 min. Injections may be given weekly or, in some cases, two times per week. After the maintenance dose of 0.5 mL of 1:100 wt/vol is administered safely several times, the dosage interval can be advanced to every 2 weeks and eventually can be extended to 4 weeks

immunodiffusion, and Western blot analysis demonstrated preserved potency of cat, ragweed, and *Dermatophagoides pteronyssinus* but significantly degraded timothy grass pollen [22]. This work suggests that while IFA WBE may be mixed with certain aeroallergens, stability was shown after only 6 months (maintenance vials may not expire for 12 months), and significant degradation was found with the single grass pollen studied. Thus, it is recommended to not mix IFA WBE with any other allergen extract.

Prevention

Protection from IFA stings is recommended in the form of close-toed shoes with socks when outdoors, avoiding sandals. In a study of dolls with and without various brands of socks whose legs were placed on recently disturbed IFA mounds, the use

of socks was shown to decrease the number of IFA that reached the lower leg above the sock in 10 seconds (1.57 vs. 5.62, $p < 0.001$) and nearly doubled the time it took IFA to reach the leg (9.24 vs. 5.1 seconds, $p < 0.001$) [23]. The use of chemical agents had no meaningful effect on IFA tendency to sting either chemically impregnated paper towels or chemically treated human skin (7.0% DEET, 20% DEET, 95% DEET, 0.5% permethrin, 100% turpentine, 100% witch hazel, sulfur, camphor, and pine tar).

Immunotherapy

IFA WBE immunotherapy efficacy was first demonstrated in a retrospective study using maintenance doses of 0.2–0.5 mL of 1:20–1:100 w/v. In this study, 47 of 65 patients on IFA WBE immunotherapy self-reported 112 IFA field stings, and only 1 (0.9% of stings and 2.1% of patients) had an SR [24]. Eleven patients in this cohort had immunotherapy recommended but declined. Six of these eleven had field stings with two suffering generalized cutaneous reaction and four suffering anaphylaxis. Skin test reactivity was decreased with IFA immunotherapy in 31 of 31 patients on follow-up, whereas reactivity did not change or worsen in 4 of 4 patients who declined immunotherapy. A sting challenge by *S. invicta* was conducted in 30 patients who completed IFA immunotherapy with only local reactions occurring in all these patients.

Rush Immunotherapy (RIT)

As IFA sting attack rates are high in IFA-endemic areas, a rapid achievement of a maintenance immunotherapy dose should be considered as opposed to a more prolonged buildup utilizing a conventional dosing schedule. IFA WBE RIT was evaluated with and without premedication in a group of 58 patients completing a 2-day protocol (Table 11.2) [25]. A 0.5 mL 1:100 w/v maintenance dose was utilized in this study. A 5.2% SR rate during the RIT protocol (3 of 58 patients) was reported with 2 on placebo premedication and 1 on study premedication consisting of terfenadine, ranitidine, and prednisone. Patients received two sting challenges 3 weeks after the RIT as a part of the efficacy evaluation arm of the study with only 1 of 56 patients reporting symptoms of an SR. This was a treatment efficacy rate of 98.2%. None of the patients reported an SR with field stings either.

Building upon the success of the 2-day RIT protocol, a 1-day protocol was reported in both children and adults. A pediatric case series consisting of three children from 22 to 36 months of age reported using a 1-day RIT protocol as safe without increased systemic effects or reactions [26]. In addition, two of these three children did not have any premedication (Table 11.3). Under a study protocol, the

Table 11.2 Two-day rapid induction of tolerance protocol [25]

Day	Volume (mL)	Concentration (wt/vol)
1	0.1[a]	1:10,000,000
	0.3[a]	1:10,000,000
	0.1[a]	1:1,000,000
	0.3[a]	1:1,000,000
	0.1[a]	1:100,000
	0.3[a]	1:100,000
	0.1[a]	1:10,000
	0.3[b]	1:10,000
2	0.1[a]	1:1000
	0.2[a]	1:1000
	0.3[a]	1:1000
	0.4[a]	1:1000
	0.5[a]	1:1000
	0.1[a]	1:100
	0.2[a]	1:100
	0.3[b]	1:100
8	0.25[a]	1:100
	0.25[a]	1:100
15	0.5[a]	1:100
22	2 IFA sting challenges	
29	0.5[a]	1:100
50	0.5[a]	1:100
Monthly	0.5[a]	1:100

[a]Sixity minutes of observation
[b]Two hours of observation

safety and efficacy of a 1-day RIT without premedication was reported in adults [27]. This study consisted of 37 patients ages 18–49, and 9 (24.3%) of those enrolled experienced an SR during the RIT. Of those experiencing an SR, four of the nine had objective symptoms including urticaria and tachycardia, and five of the nine had subjective symptoms which included generalized pruritus, dyspnea, and throat scratchiness. In an extension of the 1-day RIT for IFA WBE immunotherapy, a protocol was designed to evaluate whether premedication would reduce the rate of SR [28]. In this study, 4 of 42 (9.5%) patients experienced SR during the RIT. Premedication with twice-daily oral loratadine (10 mg), ranitidine (150 mg), and prednisone (20 mg) began 2 days prior to the 1-day RIT and was completed with the evening dose on the RIT day. Three patients demonstrated objective symptoms including flushing, rhinorrhea, and urticaria. One patient demonstrated subjective pruritus. While not statistically significant ($p = 0.07$), the SR rate was reduced from 24.3% (9 of 37) to 9.5% (4 of 42). Four of six patients who experienced reactions during skin testing (prick and intradermal) also experienced an SR during RIT: two were in the premedication group and two were not. The protocol included a single sting challenge 3 weeks after RIT. One patient with an SR to skin testing had an SR to a sting challenge but not during RIT (no premedication).

Table 11.3 One-day rapid induction of tolerance protocols [26, 27]

Day	Volume (mL)	Concentration (wt/vol)
1	0.3[a]	1:100,000
	0.1[a]	1:10,000
	0.3[a]	1:10,000
	0.05[a]	1:1000
	0.15[b]	1:1000
	0.3[b]	1:1000
	0.05[b]	1:100
	0.1[b]	1:100
	0.2[b]	1:100
	0.3[c]	1:100
8	0.25 each arm[a]	1:100
15	0.5[a]	1:100
22	IFA sting challenge*	
29	0.5[a]	1:100
50	0.5[a]	1:100
Monthly	0.5[a]	1:100

[a]Observation time 30 min
[b]Observation time 60 min
[c]Observation time 2 h
*Only in reference [19]

The single patient who had a reaction to the skin testing but tolerated RIT without reaction was in the premedication group. Fifty-four of 66 who completed RIT consented to sting challenge, one had an SR as previously noted, and one presented for a sting challenge but the ant would not sting and was later identified as *Crematogaster laeviuscula*. This patient later demonstrated field stings without reaction.

Systemic Reactions to Testing and Treatment

Seventy-seven patients who received IFA conventional immunotherapy were reviewed as a part of a retrospective case cohort study [29]. This study found an SR rate of 0.42% per injection and 9.1% per patient. A total of five SRs had an onset within 30 min after the injection which were treated successfully with injectable epinephrine. Three of the SRs were self-reported by patients after leaving the office and resolved without medical intervention. In addition to these SRs, LLRs developed in 1.1% of injections and 20.7% of patients. SRs were seen in buildup phase in 2.9% of injections and occurred in 3.9% of injections while on maintenance ($p > 0.05$). SR were seen in 9.5% of skin tests. Increased odds ratios were seen with SRs to immunotherapy in patients experiencing LLRs to IFA stings (OR 34.4; 95% CI; 6.52–18.2) and in those who experience SRs to skin testing (OR 4.75, 95% CI, 1.13–20.0).

Rarely, a patient may demonstrate SR to IFA WBE immunotherapy. In those patients, while not FDA approved and considered off-label, omalizumab has been described as adjunctive therapy with immunotherapy or used as sole therapy. A patient who presented for a history of allergic rhinitis and five SR to IFA (immediate-onset urticaria, globus, rhinitis, and asthma symptoms) demonstrated IFA WBE sensitivity (intradermal, 1:20,000 wt/vol dilution, 5 mm wheal, and 30 mm flare) [30]. After beginning aeroallergen and IFA WBE immunotherapy, she twice developed SR (ocular pruritus, peak flow decrease 9–12%). This patient began omalizumab (150 mg subcutaneously once monthly) and continued immunotherapy for IFA WBE and aeroallergens separated by at least 24 h. After two omalizumab doses, she had an SR to IFA WBE immunotherapy. Admitted to an intensive care unit after three doses of monthly omalizumab therapy, the patient tolerated 2-day IFA WBE RIT with premedication, developing ocular pruritus and injection on the first day but no ocular symptoms when olopatadine was given prior to the second day RIT. The patient completed 15 months of IFA WBE immunotherapy with monthly omalizumab without SR. In a single case report of a patient who experienced SR to both field sting and IFA WBE immunotherapy in whom IFA WBE immunotherapy was discontinued, omalizumab was shown to be protective against IFA SR during sting challenges and field stings [31]. At the time of the report, the patient had received monthly omalizumab for 70 months without SR to sting challenge and field stings but did not describe future plans for reevaluation or discontinuation of therapy.

Compliance with Therapy

While IFA WBE immunotherapy reduces the risk of SR to subsequent stings, patients may be hesitant to start or continue immunotherapy. In a prospective study of 76 patients for whom IFA WBE immunotherapy was prescribed, 54 (71%) consented and started IFA immunotherapy, and at 1-year follow-up, 26 remained on IFA immunotherapy (35% of 74 successfully contacted) [32]. In the 1-year follow-up interval, ten patients reported having moved away, but of those, six remained on IFA immunotherapy. Over half cited inconvenience as a reason for nonadherence to IFA immunotherapy and over a quarter cited needle phobia at the initial and follow-up evaluations. In a prospective study, patients who had received IFA WBE immunotherapy were followed after completing 15–240 months of maintenance immunotherapy [33]. Immediately after stopping immunotherapy, none of the 30 reported SR to field stings and 4 of 30 had evidence of IFA sensitization by skin testing. After an average of 18 months later, 12 of 15 who were skin tested showed IFA sensitization, and none of the 15 reported reaction to field stings. After an average of 57 months after stopping immunotherapy, eight of nine who were skin tested showed sensitization, and none reported reaction to field stings. In a retrospective chart review and a survey of patients who received IFA immunotherapy and had

discontinued, only 34 of 272 were available for comparison. Among two groups who had received either less than (suboptimal) or at least 3 years (recommended) of maintenance IFA immunotherapy, a low incidence of SR following IFA immunotherapy discontinuation occurred in both groups with one reaction in each (6% vs. 7%). The suboptimal group received a mean 1.47 years of therapy and all but one experienced a subsequent sting (19 patients), while those who completed therapy as recommended had a mean 5.53 years of therapy with 14 subsequently stung (15 patients) [34].

Range and Other Species

SR to "fire ant" has been reported as far north as Toronto, Canada, but it is not clear if the stinging insect was *S. richteri*, *S. invicta*, or another stinging ant [35]. Reporting an SR to a stinging red ant in a Toronto park, a 43-year-old woman with history of travel to Florida and other southern states in the USA was found to have skin prick sensitivity to IFA WBE (6 mm wheal) but negative skin prick and intradermal testing to flying Hymenoptera. It is likely that her reaction was to *Myrmica rubra*, described as infesting areas of the northeast US and Southeastern Canada [36]. *M. rubra* is native to the Palearctic north from western Europe through western Siberia and described as the most aggressive *Myrmica* species. First described as a stinging insect in the 1960s and 1970s in Maine, reports increased from 1993 to 2003. Nest density may reach as high as four nests per square meter with an average of 1.24 in Acadia National Park in July. Introduced from Europe, it has been found in Rhode Island, Massachusetts, coastal New Hampshire and Maine, and New York and in Quebec and Ontario, Canada. Its behavior is similar to the IFA, described as aggressive, with nests in places with high humidity near the soil such as tree or shrub roots, under rocks or logs, and in decaying logs [37]. They may have 0.5–1.5 nests per square meter. Disturbance of their nests leads to stinging behavior, resulting in both local symptoms of wheal and flare and systemic anaphylaxis. Notably, the *Myrmica* genus is within the Myrmicini tribe, a subfamily of Myrmicinae, similar to the *Solenopsis* genus, of the Solenopsidini tribe also in the Myrmicinae subfamily of the Formicidae family [38]. That both *Solenopsis* and *Myrmica* genera are within the Myrmicinae subfamily of the Formicidae family would suggest their venoms may have cross-reactive antigens as do the individual species within the *Solenopsis* genus and could account for the Toronto patient's SR to a local ant with evidence of IFA WBE sensitization. Should significant cross-reactivity exist, determining if IFA WBE immunotherapy could be used to prevent anaphylaxis to *M. rubra* stings is a possible avenue of research.

The IFA's grip on the American Southeast may be threatened by another invasive ant species. *Nylanderia fulva* invaded Houston, Texas, in 2002 and was also reported in Florida. This ant was previously reported as *N. pubens* and Raspberry crazy ant and has expanded its habitat to include Texas, Louisiana, Mississippi, and Florida [39]. Their expansion is associated with a decline or elimination of *S. invicta* species

in examined sites. Because *N. fulva's* environmental tolerance of cold is unknown, its potential range cannot be predicted. *N. fulva* ants compete with *S. invicta* ants by usurping food and displacing *S. invicta* from their nests. While *S. invicta* attempts to attack *N. fulva* ants with the same venom so toxic to humans, an *N. fulva* ant detoxifies itself with its own exocrine gland secretions leading to 98% survival after attack (only 48% of ants whose exocrine glands were blocked by nail polish survived, $p < 0.0001$). The exocrine gland secretion responsible for inactivation of *S. invicta* venom is concentrated formic acid, which may denature the protein fraction of *S. invicta* venom causative of anaphylaxis in humans. This data raises the possibility that a topical agent (some non-toxic preparation of formic acid) applied to human skin could act as a shield against the toxic effects of IFA venom [40].

Conclusion

The IFA has a significant impact upon those visiting or residing in endemic areas in the southeastern USA. Two percent of IFA sting victims may suffer life-threatening anaphylaxis in endemic areas. Sting attack rates range from 38 to 51% with the pediatric population often at disproportionate risk. Children also demonstrate a significantly greater prevalence of IgE sensitization to IFA than adults. IFA hypersensitivity is evaluated with WBE skin prick and intradermal testing and, if negative, serologic evaluation for IFA-specific IgE. Those patients with IFA hypersensitivity should receive autoinjectable epinephrine appropriate for weight and age. IFA immunotherapy is recommended for children and adults who have SR. The duration of therapy typically mirrors that of the flying Hymenoptera but may be continued indefinitely depending on the severity of their initial reaction and the risk of future reactions. IFA immunotherapy maintenance dosages may be safely achieved utilizing a 1- or 2-day RIT schedule as those living in endemic areas are at a high risk for future stings.

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Chapter 12
Mosquito Hypersensitivity: Clinical Updates

Renata J.M. Engler and Howard C. Crisp

Introduction

Mosquitoes, among the most common biting insects, are part of the vast invertebrate phylum of arthropods that includes gnats and flies (also six-legged) as well as the eight-legged spiders, mites, and ticks. Classified within the Order Diptera and Family Culicidae, over 3700 species of mosquitoes represent major global vectors for bite transmission of diseases causing significant mortality (estimated more than one million deaths per year) and morbidity related to malaria, yellow fever, Japanese encephalitis, dengue fever, chikungunya, West Nile virus, Rift Valley fever, St. Louis encephalitis, eastern equine encephalitis, western equine encephalitis, Zika virus, *Mycobacterium ulcerans*, and various other arboviruses [1–4].

This chapter will focus on the clinical, immunologic, diagnostic, and therapeutic aspects of mosquito bite hypersensitivity, a common affliction for both children and adults [5, 6]. Typically manifesting as immediate and/or delayed local reactions, the impact on quality of life and particularly outdoor activity may be profound, particularly in the setting of large local reactions and when systemic symptoms are also present [5–13]. Large local reactions may be so severe that they are mistaken for a skin infection [11–15]. Severe local reactions such as Skeeter

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R.J.M. Engler, M.D. (✉)
Department of Medicine, Allergy-Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Department of Medicine, Walter Reed National Military Medical Center (WRNMMC), Bethesda, MD, USA
e-mail: Renata.Engler@gmail.com

H.C. Crisp, M.D.
North Texas Allergy & Asthma Associates, Dallas, TX, USA

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syndrome and systemic reactions including generalized urticaria, angioedema, and anaphylaxis have been reported [9–17]. Thankfully, systemic allergic reactions appear to be very rare, considering the large number of mosquito bites that occur daily worldwide. Young children, immune deficient persons, and immigrants or visitors to an area with "new" exposure to indigenous mosquitoes may be at increased risk for more severe reactions [18, 19]. Some individuals, due to underlying clinical risk factors, may have profoundly enhanced reactions, such as in individuals with agammaglobulinemia [20], chronic human immunodeficiency virus (HIV) infections [21, 22], and chronic Epstein-Barr virus (EBV) disorders (e.g., natural killer/T-lymphoproliferative diseases) [23–31]. Table 12.1 outlines the spectrum of mosquito bite reactions, symptoms, and signs as well as timing of peak reaction.

Table 12.1 Spectrum of mosquito bite reactions

Reaction type	Symptoms	Signs	Peak after bites
Local, common	Itching, burning	White and reddish bumps, local swelling	~20 min
	Itching, soreness	Hard, reddish brown bump, or multiple bumps (indurated papules)	24–36 h → may last days or weeks
	Itching, burning, soreness	Small blisters instead of hard bumps, may be with ecchymosis (bruising, dark spots)	4–6 h, persisting for days or weeks
Local, severe/large	Itching, burning, swelling over large areas	Angioedema, redness	Within minutes usually less than 2 h
	Localized pain (may have systemic symptoms)	Ulceration increasing	Over days to weeks (may be due to venulitis or secondary infection such as *Mycobacterium ulcerans*)
Skeeter syndrome	Large local inflammatory reaction, low-grade fever	Often misdiagnosed as cellulitis	Over days to weeks
Systemic reaction, with local reactions	Fevers, headache, body aches, low-grade fever, swollen lymph nodes	Signs of infection and/or severe immune inflammatory reaction, hepatosplenomegaly, hematologic abnormalities	After several hours, may last days Rule out: Underlying chronic infection (HIV, EBV), consider *Mycobacterium ulcerans* and risk for lymphoproliferative malignancies
Immediate hypersensitivity, cutaneous	Generalized hives	Cutaneous urticarial and/or angioedema	Immediate, within minutes but may flare in hours (late phase)

Table 12.1 (continued)

Reaction type	Symptoms	Signs	Peak after bites
Immediate hypersensitivity, systemic	Swelling, hoarseness, difficulty swallowing, nausea, vomiting, shortness of breath, asthma, light-headedness, collapse, cardiovascular collapse	Systemic anaphylaxis including hypotension, bronchospasm, hypoxia, cardiovascular collapse	Immediate, within minutes but may flare in hours
Inhalation hypersensitivity only	Nasal, ocular, and respiratory symptoms	Rhinoconjunctivitis, asthma	Immediate and/or delayed presumably due to inhalation of mosquito particulate antigens in the air

Mosquito Bite Hypersensitivity Reactions: Clinical and Immunologic Features

Local Reactions

Mosquito bites commonly cause cutaneous local wheal and flare reactions (between 2 and 10 mm in diameter) peaking at 20 min and delayed pruritic indurated papules peaking at 24–36 h, diminishing over several days or weeks and with no evidence of systemic effects [5, 6]. Large local reactions (often more than 30 mm in diameter) are associated with immediate onset (minutes) of itchy, erythematous swelling, and induration evolving into firm papules that can become ecchymotic, vesiculated, blistering, and/or bullous usually within 2–6 h after the bites and persisting for days or weeks. Clinical guidelines from the American Academy of Allergy, Asthma and Immunology have suggested that only the more impacting large local or systemic reactions should be considered "mosquito allergy" [32]. When associated with systemic symptoms and ecchymotic or vesicular local changes, the large local reaction may be part of an Arthus reaction (immune complex mediated) [14, 16, 32, 33]. The term "Skeeter syndrome" is defined as mosquito saliva-induced large local inflammatory reactions accompanied by low-grade fever, a reaction that is often misdiagnosed as cellulitis [14, 16]. If lesions persist and become ulcerated, consideration of bite transmitted indolent infections with Mycobacterium ulcerans must be considered [34–36].

Immediate onset large local reactions (wheal and flare) following mosquito bites appear to be IgE mediated with specificity for mosquito salivary proteins. Reunala et al. demonstrated heat-sensitive passive transfer of this type of cutaneous

hypersensitivity as well as in vitro basophil histamine release with mosquito salivary proteins (from *Aedes communis*) using passively sensitized basophils from otherwise nonreactive subjects [37]. Mosquito saliva-specific IgE was also demonstrated on immunoblotting to multiple components. Peng et al. studied 30 subjects with local reactions to mosquito bites (23 with immediate and delayed, 6 with immediate, and 1 with delayed only) and 11 subjects with no bite reactions [5]. The mean mosquito-specific IgE and IgG concentrations were highest in those subjects with immediate local reactions. There was a direct correlation between the size of the local reaction and the levels of IgE and IgG, suggesting that both IgE and IgG immune complex mediated hypersensitivity mechanisms may be involved in cutaneous hypersensitivity reactions [5]. Anti-saliva IgE and IgG4 antibodies are common in mosquito bite reactive individuals, but histamine and leukotriene C4 release appears to be involved in the early phases of response [38].

Non-IgE-mediated mast cell activation with degranulation of mediators may be involved in some mosquito bite-associated allergic reactions. Mosquito bite-induced cutaneous mast cell degranulation with tissue edema and neutrophil recruitment has been described in a murine model [39]. *Anopheles stephensi* saliva directly induced connective tissue-type but not mucosal-type mast cells to degranulate in vitro in the absence of IgE, providing the signal for rapid T cell enrichment of the draining lymph nodes with sequestration from the circulation rather than local proliferation. The response is aborted in mast cell-deficient mice, further supporting the concept that cutaneous mast cells play an important role in inducing T cell and dendritic cell recruitment within draining lymph nodes with subsequent T and B lymphocyte priming [39]. It is also noteworthy that mosquito bite saliva components have been shown to control the magnitude of Ag-specific immune responses via mast cell-mediated inflammatory responses. Using a mast cell knock-in mouse model, investigators have shown that mosquito bites consistently induced MIP-2 in the skin and IL-10 in draining lymph nodes, thereby downregulating Ag-specific T cell responses by a mechanism dependent on mast cells and mediated by IL-10 [40]. How these immunomodulating pathways stimulated by mosquito saliva impact on infection transmission and hypersensitivity reactions in humans remains to be defined.

The immune responses that correlate with delayed-onset mosquito bite local reactions include saliva protein-specific IgE and IgG levels, similar to immediate reactions, but also correlate with lymphocyte responses and delayed cutaneous reactions to skin testing [16]. In vitro mosquito antigen-specific lymphocyte proliferative responses were highest in those subjects with delayed local reactions, and the degree of response correlated to the size of the delayed local reaction. Severe delayed-onset cutaneous reactions to the point of scar formation have been described as a purely cell-mediated immune response (delayed-type hypersensitivity or DTH) in a child with congenital agammaglobulinemia and absent IgE (<0.2 IU/ml) [20]. Seasonal bullous eruptions have been linked to mosquito bites in one case report from England [18]. Delayed-onset cutaneous hypersensitivity reactions may result in prolonged papule formation, and it is noteworthy that mosquito whole-body extract has been described as a potentially useful delayed-type hypersensitivity

(DTH) skin test antigen. One study of 321 normal volunteers demonstrated 19% with a classic DTH skin test response to mosquito extract [19].

In a study of serial skin biopsies following mosquito bite delayed large local reactions by Karppinen et al., the inflammatory cell response was similar to that seen in late-phase allergic responses with increasing dermal eosinophils and neutrophils within 2 h after bite exposure and increasing mononuclear and CD4+ cells at 6 and 24 h [41]. While clinically effective for prophylaxis of mosquito bite large local reaction symptoms, pretreatment with cetirizine (a selective inhibitor of peripheral histamine) in this double-blinded experiment was associated with increased dermal accumulation of eosinophils, mononuclear cells, and CD4+ cells compared to the placebo-treated group at the site of the bite reactions [41].

Most of the population at any given time will have some local reactivity to mosquito bites. Immediate reactions occur in 70–90% and delayed reactions in 55–65% of patients subjected to mosquito bites [5, 13, 42]. In a cross-sectional survey study of over a thousand school age children (11 schools, Monterrey, Nuevo Leon, Mexico), seventy-six percent reported adverse reactions to mosquito bites in the previous 12 months with itching (75%) and rash (72%) being the most frequent symptoms [43]. In a survey of 482 patients ranging from age 2–54 years (mean 18.5 +/− 14.8 y), the incidence of self-reported large local reactions was 2.5%, with the majority younger than 10 years of age [42].

Spectrum of Local to Systemic Reactions: An Evolution of Immune Responses

Although mosquitoes bite billions of people around the world every year, reports of systemic anaphylaxis remain relatively rare. Since the early 1900s, studies of the more common local reaction patterns from early childhood to adulthood have consistently reported a staged pattern of local sensitization/reactivity that is summarized below (Table 12.2) [44–46].

Every individual does not necessarily experience all phases of these reactions. The initial period of nonreactivity has an estimated period of induction or sensitization lasting 11–12 days, with some remaining in Stage 1 for prolonged periods. In countries with mosquito swarming periods, children with severe and

Table 12.2 Stages of mosquito bite reactivity

Stage 1	No reactivity
Stage 2	Delayed localized skin reactivity
Stage 3	Biphasic local reaction including immediate and delayed inflammation
Stage 4	Immediate wheal and flare reaction only
Stage 5	No reactivity

persistent local reactions may be severely restricted in their outdoor activity because of cumulative bite morbidity [44].

Fortunately, current understanding of the natural history of mosquito bite sensitization supports a natural desensitization process in the majority of individuals [47]. It is noteworthy in Dr. Peng's 2004 study (402 children exposed to mosquito bites) that boys had higher mean mosquito saliva-specific IgG levels than girls. IgE levels peaked at the age of 6–12 months of age, and IgG levels peaked at 1–6 months of age. Levels of IgE and IgG antibodies gradually declined after the age of 5 years. A prospective study in a healthy individual exposed for the first time to 100 bites (*Culex quinquefasciatus*) followed by repeated exposures every 2 weeks for 10 months demonstrated the progression from no skin reaction through immediate and delayed induration responses (and back to nonreaction) that paralleled rise (and fall) in salivary specific IgE and IgG (with correlation to size of induration) [48]. This study suggested that both IgG and IgE play a role in the hypersensitivity response. In contrast, in the rabbit part of the study, IgG appeared to act as a blocking antibody.

Systemic Reactions: Anaphylaxis and beyond

Immediate hypersensitivity systemic reactions following mosquito bites include angioedema, generalized urticaria, nausea, vomiting, diarrhea, wheezing, and other cardiovascular manifestations of anaphylaxis. These reactions are reported rarely and there are no published reports of a possible anaphylactic death [17, 37, 49, 50]. There may be an increased risk for mosquito bite systemic anaphylaxis in patients with mast cell disorders. Reiter et al. described a 56-year-old man in Germany who had progressively worsening mosquito bite-associated cardiovascular and gastrointestinal anaphylaxis, ultimately resulting in cardiovascular arrest complicated by disabling hypoxic brain damage and tetraplegia [51].

There have also been reports of severe anaphylaxis with the wasp-mosquito syndrome associated with clinical sensitization to wasp venom, mosquito bite saliva, and, finally, horsefly bites. There is serologic evidence of cross-reactive sensitizations using an in vitro serologic immune inhibition technique between wasp venom and mosquito saliva proteins as well as horsefly bites [52, 53]. The shared allergen was characterized by electrophoresis and immunoblot as most consistent with a common hyaluronidase (MW of 44KD) [52]. The wasp/mosquito syndrome may be more common than has been previously appreciated and may play a role in some cases of idiopathic anaphylaxis and first bee sting hypersensitivity reactions [51–53].

Recurrent serum sickness-like reactions to mosquito bites have been described [54]. A 62-year-old man with a 20-year history of recurrent delayed-onset illness starting about 6 h after mosquito bites included a symptom complex of malaise, chills, fever, headache, cervical lymph node enlargement, arthralgias, myalgias, generalized purpura, and leukopenia. The presence of both mosquito salivary

protein-specific IgE and IgG was considered as a biomarker of the immune responses potentially contributing to the clinical manifestations.

Multiple reports have described patients with severe hypersensitivity to mosquito bite (HMB) including unique features characterized by bulla formation (evolving to variable necrotic changes) with intense erythema on mosquito bitten sites, eschar healing as well as frequent systemic symptoms such as fever, lymphadenopathy, and hepatosplenomegaly [29, 55–58]. Reported in predominantly Asian populations (particularly Japanese heritage), this clinical presentation (in both children and adults) has been repeatedly associated with chronic (active) Epstein-Barr virus (EBV) infections (particularly with chronic lymphoproliferative syndromes) and increased risk for serious life-threatening malignancies [29, 55–64]. The malignancies frequently involve T/natural killer (NK) cell leukemias or lymphomas, primarily mediated by EBV-infected (monoclonal or oligoclonal) NK cells [29, 57–63].

In 2012, the lymphoma workshop at the European Association for Hematopathology (EAHP) proposed a revised classification for EBV T/NK cell lymphoproliferative disease (LPD) with chronic active EBV infection (CAEBV) as an umbrella term that covers the systemic and cutaneous forms including hydroa vacciniforme (HV)-like lymphoma and mosquito bite hypersensitivity [29]. EBV-associated/NK LPD has been primarily reported in Asians and Native Americans with linkage to 15% of non-Hodgkin's lymphoma in southwest region of China; 6.1% in Korea; 7.8% in Guatemala; 2.6% in Japan; 2–3% in Taiwan, Peru, and Chile; but less than 0.1% in Europe and North America [29]. It has been estimated that around 70% of CAEBV patients present with an episode of hypersensitivity to mosquito bite (HMB) and have the potential of developing fulminant hemophagocytic lymphohistiocytosis (HLH), a potentially life-threatening EBV-triggered immune inflammatory disease that represents a subset of all HLH disease (hereditary and sporadic) [61, 62]. Lee et al. [62] described the immunologic differences between HMB and HLH associated with EBV: HLH episodes were characterized by decreased perforin expression with predominant CD3+ EBV virus load distinct from HMB episodes with predominant NK EBV virus load. The possible role of non-elevated memory CD4+ cells and/or activated lymphocytes (CD2 + HLADR+) as markers of increased mortality risk with HLH was postulated. Using an EBV-infected NK cell line from a patient with HMB, Suzuki et al. [65] demonstrated that the majority of the cells were negative for perforin, major histocompatibility complex class I-restricted NK cell receptors, CD94 and KIR2D, and an activating receptor, NKG2D. These NK cells were also high secretors of tumor necrosis factor- α. NKG2D expression could be stimulated with IL-2. Seo et al. [66] demonstrated disordered expression of inhibitory receptors on the NK1-type NK leukemic cells (IFN-gamma and IL-10 producing in response to IL-2) from three patients with HMB. More than 98% of NK cells from patients bore CD94 at a higher level than did normal NK cells with no expression of the immunoglobulin-like receptor. It is noteworthy that HMB skin reactions are characterized by an infiltration of the EBV-infected NK cells (rare in healthy individuals where EBV is largely limited to B cells) and frequently have lymphocytosis with high peripheral blood EBV DNA loads. In addition to the presence of mosquito saliva protein-specific IgE, HMB

patients have evidence of basophil activation responses to mosquito extract suggesting that basophil/mast cell mediator release is also involved in the evolution of the more severe skin reactions to mosquito saliva proteins [67].

Clinically, severe local (particularly if ulcerated) mosquito bite reactions with non-anaphylactic systemic symptoms should be carefully evaluated and followed for the early diagnosis of lymphoproliferative malignancies, particularly in Asian and Native American populations.

Less well characterized in the literature is the fact that aeroallergens derived from mosquito body parts may be a cause of inhalation allergy manifesting as rhinoconjunctivitis and asthma, representing cohorts that might benefit from immunotherapy [68–71]. Further research is needed to better characterize this population of patients distinct from mosquito bite reactions.

Mosquitoes, their Bites, and Salivary Proteins: Global Diversity and Complexity

Research continues on a global scale to improve our understanding of mosquitoes, their disease-carrying abilities, and the components of their saliva in relation to both the induction of hypersensitivity reactions and their immunomodulating properties [68–87]. There are more than 15,000 species (within 14 orders of arthropods and over 400 different genera) that evolved to feed on blood from warm vertebrate animals [87]. Three subfamilies are recognized within the family Culicidae, namely, Toxorhynchitinae (1 genus), Anophelinae (near 400 species), and Culicinae (greater than 3000 species). Some of these species are more clinically relevant than others because they affect humans, directly or indirectly. Table 12.3 details the taxonomical classification and distribution of the main mosquito genera and species associated with mosquito allergy (including those to which sensitization has been demonstrated in vivo/in vitro), including but not limited to *Aedes aegypti*, *Aedes vexans*, and *Culex quiquefasciatus* [4, 68]. The process of defining and considering redefinition of classifications continues to evolve with ever increasing number of species and less change in genera and subgenera over time [4].

While the focus of investigations has targeted the negative aspects of mosquito bites and mosquito saliva contributing to disease transmission, a recent study by Sales-Campos et al. [85] suggests that mosquito salivary gland extract (SGE) may be an important source of therapeutics in future research. Using oral administration of SGE from *Aedes aegypti* in an experimental model of C57BL/6 male mice with dextran sulfate sodium-induced colitis, Sales-Campos et al. were able to show improvement in clinical disease outcome and postmortem scores coupled with systemic reduction in peripheral blood lymphocytes but no adverse impact on bone marrow or other hematologic measures (e.g., macrophage toxicity). Evidence of local diminished inflammation along with reduced tissue measures of IFN-γ, TNF-α, IL-1β, and IL-5 cytokines suggested that SGE might be an important source of novel therapies for inflammatory bowel disease. In addition, the studies suggested

Table 12.3 Taxonomy and distribution of main mosquito species associated with allergy (including those to which sensitization has been demonstrated in vivo and in vitro (Adapted from Cantillo et al.) [68, 86]

Subfamily	Genera	Subgenera/ species n/n	Distribution	Species associated with allergy
Anophelinea	*Anopheles*	7/455	Cosmopolitan	*A. stephensi, A. minimus, A. sinensis*
	Bironella	3/8	Australian	
	Chagasia	–/4	Neotropical	
Culicinae Aedeomyiini	*Aelcomyia*	2/6	Afrotropical, Oriental, Australasian, Neotropical	
Aedini	*Aedes*	23/363	Old World, Nearctic	*A. aegypti, A. vexans, A. communis, A. togoi, A. albopictus, A. triseriatus*
	Ochlerotatus	22/550	Cosmopolitan	*O. triseriatus, O. hendersoni*
Culicini	*Culex*	23/763	Cosmopolitan	*C. quinquefasciatus, C. tritaeniorhynchus, C. pipiens, C. pipiens pallens, C. tarsalis*
	Deinocerites		Neotropical (mostly)	
	Galindomyia		Neotropical	
	Lutzia		Afrotropical, Oriental, Neotropical, Palearctic oriental, Australasian,	
Culisetini	*Culiseta*	7/37	New World, Nearctic	*C. inomata*
Ficalbiini	*Ficalbia*	–/8	Afrotropical, Oriental	
	Mimomyia	3/44	Afrotropical, Oriental, Australasian	
Hodgesiini	*Hodgesia*	–/11	Same as above	
Mansoniini	*Coquillettidia*	3/57	Old World, Neotropical	
	Mansonia	2/23	Same as above	
Orthopodomyiini	*Orthopodomyia*	–/38	Afrotropical, Nearctic, Oriental, Neotropical, Palearctic	

(continued)

Table 12.3 (continued)

Subfamily	Genera	Subgenera/ species n/n	Distribution	Species associated with allergy
Sabethini	*Isostomyia*	–/4	Neotropical	
	Johnbelkinia	–/3	Neotropical	
	Kimia	–/5	Oriental	
	Limatus	–/8	Neotropical	
	Others			
Toxorhynchitini	*Toxorhynchites*	4/88	Afrotropical, Australasian, Neotropical, Oriental, Palearctic Oriental	
Uranotaeniini	*Uranotaenia*	2/265	Afrotropical, Oriental, Australasian, Neotropical	

that early exposure to mosquito saliva may provide components able to prevent disease relapse. High-performance liquid chromatography (HPLC) experiments pointed to a major SGE pool fraction (F3) able to ameliorate disease signs. In the setting of systemic inflammation and atherosclerotic cardiovascular disease, anophelene saliva protein derived from the malaria vector mosquito has been identified to contain a platelet adhesion inhibitor, potentially representing a novel therapeutic drug candidate [88].

The complexity of SGE components and biologic activity drives ongoing research in mammals in order to better define immune response mechanisms. A 2016 study by Barros et al. [89] characterized a mixed-type allergic response in *A. aegypti* mosquito bite-sensitized BALB/c mice following intranasal SGE antigen challenge versus phosphate-buffered saline. Bronchial alveolar lavage (BAL) recovered from mice challenged with SGE showed an increased number of eosinophils and Th1 cytokines (IL-4, IL-5, and IL-13). Peribronchoalveolar eosinophil infiltration, mucus, and collagen in lung parenchyma (suggesting a Th2 response) were not associated with tracheobronchial hyper-reactivity. In contrast, the antibody profile in serum of SGE challenged mice versus controls evidenced a mixed-type response of both IgG1/IgE (Th2 related) and IgG2a (Th1 related) isotypes. Different from most allergy models, the exposure to mosquito bites followed by SGE intranasal challenge leads to a Th2 response in the lung environment without changes in respiratory patterns (bronchial hyper -reactivity) and a systemic mixed-type (Th1/Th2) reaction [89]. Further research is needed and may clarify the mechanisms of why mosquito bite anaphylaxis is so rare and the mechanisms behind natural desensitization.

Characterizing clinically relevant mosquito saliva allergens is further challenged by the fact that there may be regional differences in major mosquito allergens between different regions of the globe as well as between extract types. Wongkamchai et al. evaluated the major allergens of four different mosquito species utilizing

pooled sera from mosquito bite-allergic patients in Thailand. They found that using saliva only extracts revealed more allergens than salivary gland extracts, which in turn revealed more allergens than whole-body extracts. They also observed these allergens to be different from ones previously reported in mosquito-allergic patients from Manitoba [73].

Mosquito Allergens and Diagnosis

Mosquito saliva contains a complex array of biologic substances including lysozymes, antibacterial glucosidases, anticoagulants, antiplatelet aggregating factors, and vasodilators. The large protein fraction within the saliva contains known as well as potentially unidentified allergenic proteins [16, 90–102]. Many of these components can be classified according to their functionality as detailed below:

- Anticlotting and antiplatelet
- Vasodilators
- Affecting parasite transmission by arthropod vectors
- Enzymes associated with sugar digestion
- Lysozyme, which may control bacterial growth in the stored blood (mosquito's crop)
- Immunomodulators

Table 12.4 illustrates several of the mosquito saliva allergens found in *A. aegypti* along with the frequency of positive skin or serum-specific IgE testing in a population of mosquito bite-allergic individuals [73, 74, 92–101].

Reports about the components of mosquito saliva evolved from around 20 proteins (identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis for *A. Aegypti* in 1987) to descriptions of 55 proteins coded by salivary gland-specific transcripts by 2007 [5, 16, 90–99].

Table 12.4 Examples of allergens from *A. aegypti* saliva

Allergen	Molecular weight, kDa	Functions	Amino acids, n	Reactivity frequency IgE[a]	Reactivity frequency skin test[a]
Aed a 1 [73, 92–96]	68	Antiplatelet (apyrase)	562	43%	29%
Aed a 2 [73, 75, 97–99]	37	Pheromone transport	321	11%, 43%	
Aed a 3 [93, 100]	30	Antiplatelet (aegyptin)	273	32%	
Aed a 4 [101]	67	Sugar digestion	579		
Aed a 10	32.3	Muscle contraction (tropomyosin)	284		

[a]www.allergome.org, accessed July 4, 2016

The explosion of information about allergens relevant to thousands of organisms from bacteria to mammals has resulted in the development of real-time databases to enable rapid cataloging and referencing in support of research. The Allergome (http://allergome.org) platform, launched in 2003, was developed to support classifying allergens, IgE-binding antigens and non-IgE-binding structures [103]. A search in June of 2016 identified over 70 mosquito-related allergens among varying species, which is sure to grow further over the coming years.

The recombinant molecules show IgE reactivity similar to that of the native allergens and represent improved candidates for diagnosis and treatment of mosquito allergies [6, 72, 97–101].

A 2016 study by Peng et al. demonstrated that serum IgE levels specific for recombinant Aed a 3 (rAed a 3, corresponding to the Aegyptin protein) were significantly higher in mosquito bite test-positive versus bite test-negative subjects. Using the geometric mean of controls plus two standard deviations (SD) as cutoff level, the specificity was 100% with a sensitivity of ~46%, while skin testing had a somewhat lower sensitivity (32%) at the specificity cutoff of 100% [72]. The recombinant Aed a3 protein, corresponding to the salivary Aegyptin protein, is a major mosquito salivary allergen. Its recombinant form has biological activity and is suitable for use in skin tests and specific IgE assays in mosquito-allergic individuals. However, a cautionary note for this vision is the fact that there is considerable diversity in specific IgE binding to major allergens of saliva, salivary gland, and whole-body extracts of mosquito species in Thailand when compared to findings in Canadian populations and potentially in other parts of the world [73, 74].

Further characterization of major allergens from mosquito saliva and their production through recombinant protein technology will hopefully lead to standardized diagnostic and therapeutic agents for the improved management. However, biodiversity in human populations as well as mosquito populations in different parts of the world may require the combination of multiple allergens or regional differences in allergen combinations for enhanced sensitivity and specificity in diagnostic efficacy as well as potential value for desensitization.

Management of Hypersensitivity to Mosquito Bites: Diagnosis, Treatment, and Prevention

Currently, the diagnosis of mosquito allergy is primarily based on history and relevant physical findings. Given the natural history of mosquito bite local reactions and its frequency in the population, there are no indications for in vitro or in vivo diagnostic testing unless there is a history of large local, prolonged, atypical, and/or systemic reactions [79, 102].

Currently available tests to measure mosquito bite hypersensitivity related to salivary proteins are limited in both sensitivity and specificity with only whole-body extracts licensed for diagnostic testing by the US Food and Drug Administration. Detailed in Table 12.5, these extracts are not standardized in relation to major

Table 12.5 Mosquito whole-body extracts licensed in the United States: Update 2016

Species	*Aedes taeniorhynchus*	*Aedes taeniorhynchus*	*Culex pipiens*	*Culex species*
Collection source:	Laboratory raised	Laboratory raised	Laboratory raised	Pacific Northwest
Type of extract:	Whole body	Whole body	Whole body	Whole Body
Concentration, weight/volume	1:40	1:20	1:100	1:20
Preservative %: glycerin	50% glycerin	0.2% phenol	50% glycerin	50% glycerin
Manufacturer:	Greer Laboratory	Greer Laboratory	ALK-Abelló	Allergy Laboratories
Approved for diagnostic use:	Yes	Yes	Yes	Yes
Approved for treatment use:	No	No	No	No

Adapted from Crisp and Johnson [102]

allergen content, contain multiple proteins unrelated to mosquito saliva, and may contain highly variable amounts of salivary allergens. A Canadian study from 1996 (Peng et al.) evaluated seven commercial whole-body extracts in two patients with severe skin reactions and two controls, showing wide variability in protein content and ability to elicit skin reactions [104]. Currently available products in the United States include three glycerinated and one aqueous extract. All are FDA approved for diagnostic testing, but none are approved for allergen immunotherapy.

Skin testing with mosquito extracts has limited sensitivity. Skin testing with glycerinated whole-body extract was positive in only 32% of patients with immediate reactions and 6–12% of patients with delayed reactions [92, 97]. The sensitivity of these extracts for inhalant allergies to whole-body mosquito allergens has not been characterized. While recombinant salivary proteins promise improvements in diagnostic sensitivity and specificity, they are not currently commercially available. Recombinant Aed a 1 and Aed a 2 are among the best studied. rAed a 1 is an apyrase, cross-reacting with the corresponding protein in *A. aegypti*, *A. vexans*, and *A. albopictus*. rAed a 2, belonging to the D7 family of mosquito salivary proteins, cross-reacts with the corollary protein in multiple genera, including *Aedes*, *Culex*, and *Ochlerotatus* [73, 92, 96–99]. Skin prick testing with both rAed a 1 and rAed a 2 have been studied, and testing with rAed a 1 alone may be as good or better than testing with whole-body extracts [92, 97, 102]. The data on the clinical relevance of cloned Aed a 3 allergen detailed earlier suggests that this major allergen has very high specificity for skin reactivity [72].

Clinically available in vitro testing for mosquito saliva-specific IgE is very limited. Laboratory testing for specific IgE to *Aedes communis* whole-body extract is available in the United States via ImmunoCAP (Phadia, Uppsala, Sweden) [105]. However, a study comparing a mosquito saliva-capture enzyme-linked immunosorbent assay (ELISA) to the commercial assay, found the ELISA to be more sensitive and specific in patients with systemic or severe allergic reactions to mosquito bites

[16]. Mosquito saliva-specific IgE may be detected in as many as 90% of children, but the prevalence in 1059 random blood donors was 18% [106, 107]. Cross-sectional population studies have shown similar rates of anti-mosquito IgG and IgE in most age groups after 2 years of age [107, 108].

Mosquito bite testing is considered the standard for diagnostic testing [102]. However, performance of such testing outside a research setting presents many difficulties. Obtaining a source of female insects, concerns about disease transmission, and the risk of eliciting a severe reaction make bite testing impractical for routine use [32]. Bite testing may be necessary in a research study design to confirm a reported clinical sensitivity or define the degree of improvement following an intervention. There are no reliable surrogate biomarkers to verify the history or quantify a response to therapy. If pursued, bite testing should be performed in facilities prepared to treat anaphylaxis using disease free, laboratory-raised mosquitoes and bite chambers that ensure containment of mosquitoes used during the testing [109].

Serologic testing for mosquito saliva-specific IgE and IgG/G4, while not particularly helpful for diagnosing severity of hypersensitivity reactions, appears to have value for the diagnosis of mosquito-specific exposures and is being reported for the use of immuno-epidemiologic surveillance of populations [110–112].

Prevention and Treatment

While prevention of mosquito bites is an ideal goal, complete avoidance may be very difficult for some individuals, particularly when occupational or leisure activities are linked to higher exposure risks. In addition, the female mosquito, seeking blood to develop fertile eggs, exhibits "blood-sucking preferences" ([113], Dr. Jerry Butler, PhD, Professor Emeritus at University of Florida). About 10–20% of people are highly attractive to mosquitoes, and it is estimated that 85% of individual susceptibility is genetic [114, 115]. The nature of sweat components and body odors as well as the bacterial flora on the skin all appear to contribute signals that attract mosquito bites [116, 117]. Other factors or attributes associated with increased risk for mosquito bites include the following [118–130]:

- Size related to larger surface area and CO_2 output which is a mosquito attractant [118].
- Blood group O subjects attracted more *Aedes albopictus* than blood groups B, AB, and A but only significantly more attractive in comparison to blood group A with blood group O disaccharide (H antigen) being a particularly potent attractant [119].
- Higher body temperatures, one possible mechanism of increased risk for women in pregnancy [120].
- Pregnancy associated with several risk factors [120]:
  - Advanced pregnancy associated with increased exhaled volumes (by 21%) linked with moisture and CO_2 in breath, potent attractants.

  - Abdomens of pregnant women were 0.7 °C hotter coupled with a larger surface area.
  - Possible changes in skin chemicals and microbiome.

- Alcohol in general and beer ingestion specifically are linked to increased mosquito bites [121, 122].
- Properties of skin and sweat, human body odors (more than cattle odors), and chemical composition (e.g., lactic acid, uric acid, ammonia, carboxylic acids, etc.) drive mosquito behavior and bite frequency (and are enhanced by not bathing regularly) but with evidence of geographic and species variability [117, 124–126]:
  - Applied scents such as octanol, some perfumes and contact exposure to certain volatile chemicals may also enhance attraction.
- Other observations: movement particularly with sweating, wearing dark clothing, and have not showered in several days (old sweat is more attractive to mosquitoes) [117, 129, 130].

There is currently no evidence that the content of the diet (e.g., garlic), supplements (e.g., vitamin B6), or sugar in the blood is a cause for increased attractant effects related to mosquito bite frequency [115, 127].

In addition to avoiding travel for certain at risk populations (e.g., women who are pregnant or trying to get pregnant), the Centers for Disease Control and Prevention (CDC) currently recommends the following avoidance measures [131, 132]:

- Wearing protective clothing with long sleeves and long pants (permethrin or Environmental Protection Agency (EPA)-registered insecticide).
- Lodging in rooms that are air-conditioned with screens on windows and doors.
- Sleeping with bed nets (better protection if permethrin treated, at least 156 holes per square inch and long enough to tuck under a mattress) particularly if not in spaces where keeping mosquitoes out is feasible.

Additional measures variably recommended for reducing risk of bites from mosquitoes and other arthropods include the following (Adapted from [132–134]):

- Avoid activity outside at dawn and dusk, the most active times for mosquito females seeking a blood meal.
- Wear socks and closed toe shoes.
- Loose, long sleeves and pants (mosquitoes can bit through tight clothing), tucking shirts into pants and pants legs into socks.
- Wear light colors (less visible to mosquitoes searching for a meal).
- Shower after you get home, inspect skin, and consider tossing clothes into the dryer on high heat to kill hitch-hiking insects.
- Apply repellent only on exposed skin or clothing (as directed on product label) and never under clothing.
- Don't apply repellents over cuts, wounds, or irritated skin. When applying to your face, spray first on your hands and then rub in, avoiding your eyes and mouth and using sparingly around ears.

- Don't let young children apply. Instead, put it on your own hands and then rub it on.
- Limit use on children's hands, because they often put their hands in their eyes and mouths.
- Don't use near food, and wash hands after application and before eating or drinking.
- Use just enough repellent and only as long as needed (consider duration of action and confounders that may decrease effective time such as heavy perspiration).
- Overuse of repellent may actually reduce efficacy.
- If a DEET product is used, avoid applying sunscreens after repellent application.
- At the end of the day, wash treated skin with soap and water, and wash treated clothing in a separate wash before wearing again.
- Some directions suggest using repellents (other than permethrin) on clothes, but most of the ones tested in Consumer Reports recommendations damaged leather and vinyl, and some of them stained synthetic fabrics.
- Wash repellent off your skin and launder treated clothes after use.

Insect repellents for the reduction of mosquito bite risk is a major strategy for prevention of disease transmission and potentially less exposure for those with hypersensitivity reactions. There are currently three chemical (DEET, picaridin (icaridin in the UK, IR3535) and two natural source (lemon eucalyptus and citronella) mosquito repellents available for topical application on human skin and a sixth (permethrin) with primary use for treatment of clothes, shoes, mosquito nets, and tents. Picaridin (or icaridin) represents a valuable addition to the choice of insect repellent active ingredients with several advantages over DEET: lack of chemical odor, a non-sticky or greasy feel, and lack of any damage to clothing or plastics. Like DEET it provides longer-term protection based on bite-chamber testing [135, 136]. The details regarding efficacy (against mosquito, tick, fly, and mite bites) and safety as well as preparation types (which concentrations) are detailed in Table 12.6 [136].

Not listed in Table 12.6, permethrin (3-phenoxybenzyl (1RS)-cis, trans-3-(2,2-dichlorovinyl)-2, 2-dimethyl-cyclo-propane-carboxylate) is available in sprays (0.5%) for clothes, insect nets, sleeping bags, and boots as a repellent for Anopheline (malaria) and Culicine (arbovirus) carrying mosquitoes as well as for ticks, midges, and biting flies (all +++ efficacy rating). A pyrethroid derived from dried, crushed flowers of *Chrysanthemum* spp., permethrin is not useful on skin and may be a skin irritant in some individual. Pyrethroid resistance has been identified in some mosquitoes [136].

It is noteworthy that Consumer Reports in 2016 published an evidence-based review (based on individual bite-chamber comparative testing) of available commercial products, thereby facilitating consumer—informed choices about the most efficacious products on the market [137].

Table 12.6 Available insect repellants and their efficacy, safety, and toxicity [131, 132, 136, 137]

Insect repellents	DEET	Picardin/ icaridin *(US/UK)*	IR3535	Lemon eucalyptus	Citronella
Chemical names	N,N-diethyl-3-methyl-benzamide (Formerly N, N-diethyl-m-toluamide)	2-(2-hydroxyethyl)-1-piperidine-carboxylic acid 1-methylpropyl-ester	3-[N-butyl-N-acetyl]-aminopropionic acid ethylester	p-Menthane-3, 8-diol	3,7-dimethyloct-6-en-1-al
Sources: synthesized or natural extract Formulation/ strength %	Chemical A, L, PS, W 5–100% *Not for infants < 2 months; in children use < 20%*	Chemical L, PS, W 7–20% Efficacy best at 20%	Chemical A, L, PS, W 7.5–19.7%	Extract: leaves/twigs of the lemon-scented gum eucalyptus plant (*Eucalyptus citriodora* or *Corymbia citriodora*) PS/10–40%	Extract plant oil obtained from *Cymbopogon* spp. grasses L, Bath oils, candles/0.5–20%
Efficacy: *Anopheline (malaria)*	++	++	++	+++	+
Efficacy: *Culicine (arbovirus)*	+++	+++	+++ EPA: up to 2 h	+++ EPA: up to 2 h	+
Efficacy: ticks	+	++	++ EPA: up to 3 h	+++ EPA: up to 2 h	0
Efficacy: flies, biting mites	++	+++ Up to 12 h	+++	+++	0
Toxicity	Potential neurotoxicity if applied under sunscreen, if ingested	Possible skin irritation	Causes eye irritation; potential toxicity if ingested or inhaled	Potential skin irritation in atopic individuals	Potential eye/skin irritation and allergies
Other adverse effects	May damage plastic, clothing (cotton, wool, nylon OK)	No damage to plastics or clothing	May damage plastic and clothing		May damage clothing

Table content adapted from Diaz et al. [132]
Abbreviations: *A* aerosol, *L* lotions, *PS* Pump sprays, *W* wipes

Mosquito Reaction Therapeutic Approaches

If mosquito bites cannot be avoided, either because of occupation and/or leisure activities (relevant to quality of life) or due to a heightened individualized risk, daily dosing of prophylactic nonsedating antihistamines can reduce the severity of immediate bite-induced pruritus and wheal and flare skin reactions [138–141]. Studies with cetirizine 10 mg and ebastine 10 mg showed decreased wheal sizes by 30–40% [138, 139], with loratidine 10 mg [140] in children a reduction of 45%, and with levocetirizine 5 mg a decrease of 60% [141]. Efficacy with these drugs is by no means perfect, and some studies suggest that more than histamine is involved in local reactions, with platelet activating factor (PAF) playing a role in both immediate and delayed reactions [142, 143].

The role of other home remedies for treatment of clinical symptoms related to mosquito bites has not been well characterized in the setting of a research design for comparative efficacy. Calamine lotion, cold compresses to reduce itching, and over-the-counter topical steroids are just some of the remedies proposed on different websites and news articles. In 2012, The British Drug and Therapeutics Board reviewed the available treatments for insect bite reactions, including from mosquitoes and concluded the existing recommendations were based on expert opinion with an absence of clinical trials [143]. The summary of their review of possibly beneficial interventions included the following:

- For mild local reactions, the area should be cleaned and a cold compress applied.
- Oral analgesics can be given for pain.
- A mild corticosteroid cream applied to reduce inflammation and itching.
- Large local reactions can be treated with an oral antihistamine. Nonsedating antihistamines are preferred during the day, but a sedating antihistamine can be of use at night if sleep is disturbed.
- Antibacterial treatment is not required for simple insect bites, but secondary infections should be treated with an oral antibacterial agent in accordance with local guidelines.

It is noteworthy that there was no mention of topical antihistamines, and while these are widely available, they actually present a risk of sensitization and rashes. Oral antihistamines may be sedating in some individuals, interfering with driving safety. There are no published studies of fexofenadine, a specific nonsedating antihistamine in the context of insect bites in general or mosquito bites specifically.

Studied in Europe and currently not yet available in the US, rupatadine is a potent selective long-acting histamine (H1) receptor blocker with a dual activity through interaction with specific PAF receptors which had been shown to provide dose-related suppression of the wheal and flare in response to intradermal injected histamine and PAF and showed activity against delayed (2–6 h) onset PAF-induced platelet aggregation [144]. A double-blinded, placebo-controlled, crossover study was conducted in 30 mosquito bite-sensitive adults, comparing rupatadine at a 10 mg dose versus a matched placebo. Daily morning doses of treatment were administered, and a bite

challenge was performed on day 3 [142]. There was a 5-day washout period between trials. Outcome measures in 26 subjects included the size of the 15 min wheal diameter (55 versus 106 mm^2 on placebo, 48% decrease, $p < 0.0003$). The severity of the pruritus decreased from a score of 60 (visual analogue scale, median) under placebo to 47.5 ($p = 0.019$) with no significant differences ($p = 0.263$) in adverse events.

The response of late-phase reactions from mosquito bites to prophylactic daily dosing with antihistamines has generated diversity in clinical responses further supporting the concept that mechanisms involved in these reactions may not be uniform [138–141]. The rupatadine study showed a trending toward effect in late-phase reactions but did not reach statistical significance [142]. The role of combination therapy, such as addition of leukotriene modifiers or topical glucocorticoids, deserves further study in the context of sex differences and with a double-blinded, placebo-controlled design.

Immunotherapy

There have been many reports of mosquito bite-allergic individuals, with variable results from testing and treating with mosquito whole-body extract, mostly for non-anaphylactic bite reactions. While case reports describe benefits with whole-body mosquito extract immunotherapy in hypersensitive patients, most studies have lacked either a control group or randomization [17, 145–147].

Ariano and Panzani selected 20 patients with allergic rhinitis and large local reactions to mosquito bites for treatment with subcutaneous injections of *Aedes communis* whole-body extract over 18 months. After treatment, all reported disappearance of their local reactions, while none of ten untreated controls improved [145]. Other uncontrolled studies of immunotherapy include intradermal injections of *Culex* and *Aedes* whole-body extracts to treat local reactions, with reported improvement [146, 147].

Several authors have identified mosquito-derived particles as important respiratory allergens in India [148]. In a double-blinded, placebo-controlled study, Srivastava et al. demonstrated improvement in asthma and allergic rhinitis after 1 year of immunotherapy with a *Culex quinquefasciatus* extract in 20 treated patients compared to 20 placebo controls [69].

Due to the lack of large-scale registries or studies with mosquito immunotherapy, understanding of optimum regimens and adverse events remain poorly understood. It is noteworthy that exposure to mosquito saliva may alter infectivity with malaria [149] and the virulence of West Nile virus infection [150, 151]. Of concern, one mouse study found increased fatalities from infection with West Nile Virus after immunization with a recombinant *Culex tarsalis* D7 protein [152].

Mosquito saliva and/or whole-body immunotherapy represents one of many orphan immunotherapies that each represent a population of patients whose problems create a significant impact on quality of life and wellness and whose morbidity is not adequately controlled with currently approved therapies [153–155]. Military

personnel, firefighters, foresters, and many other occupational and lifestyle choices are seriously impeded if mosquito allergy is not adequately controlled [17].

Severe life-threatening anaphylaxis was described in two soldiers during operational training which threatened their ability to continue a military career [17]. While prick skin testing at 1:20 dilutions were negative, intradermal skin test reactivity was documented at dilutions of 1:10,000 weight/volume for *Culex pipiens* and 1:100,000 for *Aedes agypti* in one patient and 1:1000 for the second patient. One of the patients developed respiratory and cutaneous anaphylaxis after an intradermal skin testing with a 1:1000 dilution of *A. aegypti* extract and had experienced recurrent anaphylaxis in the field. Ten control subjects with exposure history but no reactions were skin test negative at these dilutions. At the time, whole-body extract was still approved for immunotherapy, and the patients requested an attempt at desensitization to preserve their military career options. Rush immunotherapy starting at a dilution of 1:1 million over 3 days was initiated. One patient tolerated the therapy with excellent response and return to duty. The second patient developed clinical symptoms of serum sickness (severe arthralgias, fatigue, myalgias, weakness, and swelling of distal extremities (without urticaria)). The problems persisted with recurrent attempts (at the patient's request) resulting in termination. This experience highlighted that while rare, mosquito bite anaphylaxis can be life-threatening and may rarely require desensitization.

There remains a great need to improve understanding of the biodiversity and personalized medicine needs of patients with mosquito-related bite or inhaled hypersensitivity. The potential that immunotherapy may benefit some of these patients has resulted in the clinical off-label use of the existing extracts for immunotherapy. There is no centralized clinical registry to monitor outcomes related to these patient experiences. While research continues to improve characterization of major clinically relevant allergen, the support to build better immunotherapy remains under-resourced. Perhaps the growing interest in immunomodulatory components and role in inflammatory disease treatment may drive the future development of standardized extract components.

Currently, none of the available commercial mosquito extracts are approved for therapy in the United States. Randomized, double-blinded, placebo-controlled immunotherapy trials in patients with large local or systemic reactions are needed with objective end points. Studies using recombinant allergens as immunotherapy should be considered. Immunotherapy with any extract carries the risk of local and systemic allergic reactions, and appropriate precautions should be used. Serum sickness due to whole-body mosquito extract immunotherapy has been reported [17].

Future Directions in Research

While our understanding of mosquito allergy has progressed substantially over the past decades, many knowledge gaps exist (Table 12.7). Immunopathologic mechanisms continue to be clarified. Pharmacologic treatments for large local reactions,

Table 12.7 Potential areas of future research and development

Immunopathology	Biopsies of delayed local reactions with staining for immunoglobulins and complement
Extracts	Standardization of extracts Commercial head/thorax, salivary gland, or saliva only extracts Commercial recombinant allergen extracts Better characterize utility in epicutaneous, intracutaneous, and in vitro testing
Therapy	Randomized, double-blinded, placebo-controlled trials of immunotherapy and pharmacotherapy (leukotriene modifiers, topical glucocorticoids, etc.)

Adapted from Crisp and Johnson [102]

beyond antihistamines, should be rigorously studied. Standardized mosquito salivary or recombinant allergen extracts are needed for both diagnostic testing and potential therapies.

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Chapter 13
Reactions to Arthropod Bites

John C. Carlson

Introduction

A wide variety of arthropods bite humans, generally when seeking to feed or in response to a perceived threat. Reactions include the innate responses to the physical effects of the mouthparts, toxic effects from saliva, and adaptive immune responses against salivary components.

Bite reactions evolve over time and can broadly be separated into immediate effects (related to the trauma inflicted by mouthparts, neurotrophic venom components, and mast cell-mediated immediate-type hypersensitivity), late effects (related to necrotoxic and other venom components, eosinophil degranulation, immunoglobulin-mediated activity), and delayed reactions (innate reactions to tissue damage and venom, T-cell-mediated inflammation). These three phases are presented as an overview of bite reactions; additional details about allergic reactivity are provided later in the chapter.

Immediate Effects

Trauma

The amount of pain induced by bites is related to the morphology of the mouthparts. The serrated mouthparts of horseflies and deerflies induce significant pain, as do the shovel-shaped mouthparts of stable flies. These flies are not adapted to feeding on humans and are unlikely to complete feeding because of the bite

J.C. Carlson, M.D., Ph.D.
Sections of Allergy and Immunology and Community Pediatrics and Global Health, Tulane University School of Medicine, SL-37, 1430 Tulane Ave., New Orleans, LA 70112, USA
e-mail: jcarlso@tulane.edu

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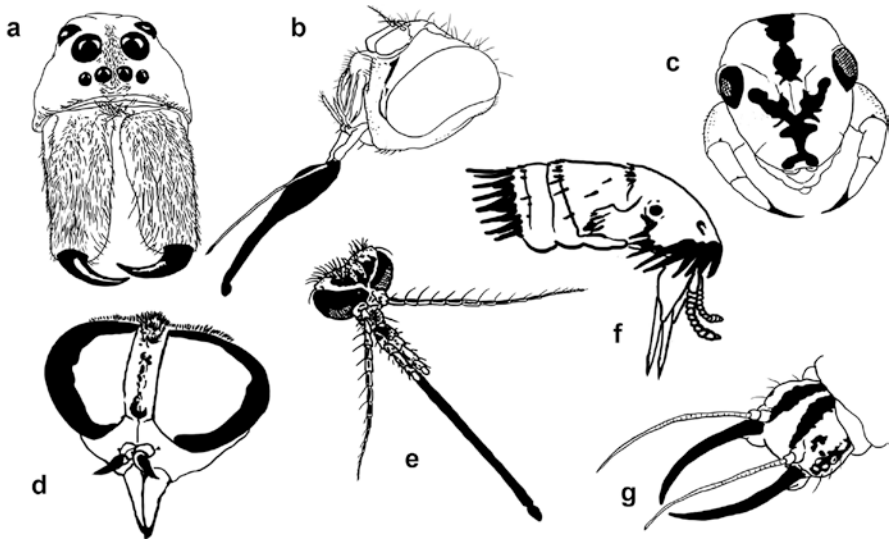


Fig. 13.1 Morphology of arthropod mouthparts. (**a**) spider, (**b**) stable fly, (**c**) centipede, (**d**) horsefly, (**e**) mosquito, (**f**) flea, (**g**) lacewing. This figure is created by Mark Fox

victim's active response to the pain. This is in stark contrast to the fine, piercing mouthparts of many anthropophilic mosquitoes that cause minimal discomfort when inserted. Injection of anesthetics makes the bites undetectable in the absence of immediate-type sensitization. Figure 13.1 depicts some of the morphological variation in arthropod mouthparts and the associated pain from the direct trauma.

Many species will attempt to bite in self-defense, puncturing or abrading the skin but otherwise injecting no saliva or injecting saliva lacking neurotoxic or necrotoxic components. Thrips, corsair bugs, and lacewing larvae seem to have a propensity to bite without provocation.

Neurotropic Venom

As with the venom from some insect stings, the venom injected by mouthparts evolved to incapacitate prey. In general the venom has evolved to work on invertebrate species and is not effective on human nerves. However some effect on human nerves is seen in the venom of several important groups.

Neurotropic components of venom have direct effects on nerves, with some species seeming to affect sensory nerves and others predominantly motor nerves. Sensory nerves are presumed affected in cases where the pain experienced is out of proportion to the amount of actual damage to the tissue. Neurotropic venom

affecting sensory nerves is best-studied for social Hymenoptera, but biting taxa, such as some species of Heteroptera, can also induce a degree of pain that suggests sensory nerve effects. In contrast, the venom from some species interferes with the function of human motor neurons [1]. Black widow spiders are of greatest medical concern for their motor neurotoxins, although the venom of bark scorpions found in the southern USA also contains effective motor neurotoxins [2]. Encounters with bark scorpions may occur in their natural habitats or by handling specimens kept as pets throughout North America [3]. Tick paralysis is the result of a neurotoxin released by gravid ticks (primarily *Dermacentor* species affecting humans), but with variability in response that makes characterization difficult. Removal of the tick leads to prompt recovery in those affected.

Immediate-Type Hypersensitivity

Immediate-type hypersensitivity to salivary components injected into skin is generally cross-reactive at the taxonomic level of genus. In general, multiple prior exposures to antigens are required for the development of immediate-type hypersensitivity. IgE secreted by plasma cells, subsequently bound to mast cells, must undergo cross-linking to trigger degranulation. Histamine is the molecule primarily responsible for vascular dilatation and extravasation of fluid into the tissue that are the hallmark of immediate-type allergic reactions.

It is not clear why some immediate-type reactions remain localized, and others become systemic. It is also not clear why some arthropod taxa are less likely to trigger systemic reactions than others, or why biting arthropods seem to trigger fewer anaphylactic events than stinging insects.

After degranulation, mast cells become transcriptionally active, producing the cytokines important for late phase reactions.

Late Phase

Late phase effects are heavily dependent upon prior sensitization, particularly for the late phase effects from mast cell degranulation. Eicosanoids (prostaglandin D2 that attracts eosinophils, basophils, and Th2 cells and leukotriene C4 that promotes vascular permeability and bronchoconstriction) and cytokines (IL-4 that promotes Th2 cells, class switching in B cells, up regulates MHC2 and TNF) are released by mast cells hours after degranulation. The subsequent activation of eosinophils and basophils produces a biphasic reaction in some patients that can exceed the magnitude of effect seen during the earlier phase of immediate-type hypersensitivity reaction.

In addition to this IgE-dependent component of the late phase, local bite reactions also involve IgG, particularly well-studied in mosquito bite reactions in the Skeeter Syndrome.

Delayed Reactions

The inflammation caused by T cells typically takes 24 h to develop, although can range between hours to multiple days. The sensitization of T cells precedes the sensitization of B cells, because the isotype switching to IgE-secreting B cells requires T-cell help. Consequently, in infants and young children, delayed reactions will be the first manifestation of sensitization. With subsequent exposures, children develop both immediate-type and delayed-type reactions to a single bite with the two types of inflammation blending together in those with late phase reactions.

The itch from delayed-type inflammation is conveyed via A-delta nerve fibers that are not histamine-responsive. This is in contrast to the unmyelinated C-fibers that are responsive to the histamine released during immediate-type inflammation. Therefore it is important to note that delayed-type itch is not responsive to histamine-blocking medications.

In contrast, T cells are very sensitive to topical corticosteroid medications. It takes hours before the corticosteroids trigger the apoptosis or re-homing of the T cells into the lymph node. IgE-mediated degranulation of immediate-type reactions is not affected by corticosteroid medications.

T-cell-mediated inflammation leads to edema of the skin along with spongiosis, as the junctions between cells are weakened. This allows for large pieces of skin to be excoriated with the itch-scratch reflex. It is possible that this apparently maladaptive neuroimmunological reflex is beneficial when arthropod-borne pathogens have been deposited into the skin, but have not yet disseminated.

Biting Arthropods

Introduction

Physicians are occasionally asked to identify the probable causes of painful or pruritic skin lesions. When the bite is witnessed, identification can be made more easily than if lesions appear without a witnessed bite. Patients may be especially distressed when lesions are thought to be from encounters made indoors. Here we review the more common biting arthropods. Table 13.1 lists causes of pruritic lesions from indoor exposures while Table 13.2 reviews causes of painful lesions from indoor exposures to serve as an aid in the differential.

Table 13.1 Common causes of pruritic papules obtained inside US homes

English name	Scientific name	Time of bite	Preferred location	Classic pattern	Other signs
Common species					
House mosquitoes	*Culex pipiens*	Evening	Lower extremities	Scattered	None
Asian tiger mosquitoes	*Aedes albopictus*	Evening, daytime	Exposed skin	Clusters	None
Cat fleas	*Ctenocephalides felis*	Day or night	Torso	Clusters	"Flea dirt"
Bed bugs	*Cimex lectularius*	Night	Exposed skin	Lines	Blood on sheets
Less common species					
Kissing bugs	*Triatoma* spp.	Night	Face	Swollen eye, lip	Liquid feces on sheets or walls
Bat bugs	*Cimex* spp.	Night	Exposed skin		Blood on sheets; bats in attic
Bird and rodent mites	Several	Day or night	Exposed skin	Bites confined to a single room	Birds in attic or outside wall, rodent infestation
Bird and rodent fleas	Several	Day or night	Exposed skin	Bites confined to an area of the house	Birds in attic or outside wall, rodent infestation
Thrips	Several	Day	Exposed skin	Bites confined to a single room	Indoor plant or vent to outdoors
Lacewing larvae	Several	Day	Exposed skin	Bites confined to a single room	Bite is usually witnessed

Mosquitoes (Culicidae)

Mosquito species range from intensely anthropophilic (feeding almost exclusively on humans) to those that are infrequently encountered by humans. A few species have spread around the world where they are important vectors of human disease, such as *Culex pipiens* and *Aedes albopictus*, while other species are very local in their distribution. This variability in exposure explains the variability in sensitization experienced by individuals.

Most species feeding on humans have evolved strategies to avoid detection while feeding and so do not cause sufficient tissue damage to draw attention. Local allergic reactions to mosquito bites are common, and this combination of frequent exposure

Table 13.2 Common causes of painful papules in US homes

English name	Scientific name	Time of bite	Classic pattern	Other signs
Common species				
Impetigo, folliculitis, furuncles	*Staphylococcus aureus*	Anytime	Scattered, recurring	Pain worsens over time, purulence
Less common species				Pain improves over time
Spiders	Many	Night	Two puncture marks	Single lesion
House centipedes and others	*Scutigera coleoptrata*	Night	Two puncture marks	Single lesion
Assassin bugs	Several	Night	One puncture mark	Single lesion
Fire ants	*Solenopsis invicta* and other species	Day or night	Clusters of papules	Vesicles appear ~24 h after stings

to species that are difficult to detect make mosquitoes the top of the differential in cases of papular urticarial of uncertain etiology.

Given the ubiquity of mosquitoes and the frequency of mosquito bites, anaphylactic reactions are surprisingly rare. In cases where anaphylaxis to mosquito saliva has been demonstrated, desensitization using whole-body extract and salivary extract has proven effective at protecting patients during subsequent exposure.

Horseflies and Deerflies (Tabanidae)

The serrated mouthparts of these flies cause significant tissue damage and pain. In contrast to the needle-like mouthparts of mosquitoes, these flies have knife-like mouthparts which lacerate the skin to draw blood which is then mopped up by the tongue. These painful bites can be confused with the pain from Hymenoptera stings. The saliva of these flies contains a potent anticoagulant, and drops of blood are commonly seen coming from the wounds. While feeding attempts are rarely successful on humans, the intensity of exposure in some outdoor environments can result in allergic sensitization. Patients with anaphylaxis from tabanid bites have been successfully desensitized [4]. Allergenic components of horsefly saliva include an antigen 5-like protein and hyaluronidase [5].

Other Biting Flies

There are zoophilic groups of flies that will bite humans when they are encountered, although these flies prefer to feed on other species. Most of the data on hypersensitivity to these flies are from horses (reviewed by Wilson [6]). Sand flies (Psychodidae:

Lutzomyia), black flies (Simuliidae), and biting midges (Ceratopogonidae) are very small flies that induce pain during the bite. Stable flies (Muscidae: *Stomoxys calcitrans*) are between tabanids and other biting flies in size, with an intensity of pain proportional to the size of the mouthparts.

Orange et al. [7] reported a case describing a woman with severe local reactions mistreated as skin infections and one episode associated Guillain-Barré syndrome and another associated with minimal change nephrotic syndrome. This case highlights the extent to which biting flies can cause significant morbidity.

In contrast to the Th2-type reactions generally attributed to bite reactions, sandfly bites also seem to stimulate Th1 delayed-type hypersensitivity which may play a role in protection from *Leishmania* infection [8–10].

Thrips

Thrips are very small insects that readily bite when they come into contact with human skin for reasons that are not clear. They thrive in outdoor environments, but can be drawn indoors through vents and occasionally are encountered on houseplants. Goldstein [11] describes asymptomatic bite reactions in Hawaii, and sensitization from repeat exposure is rare.

Lacewing Larvae

As larvae, lacewings are small, voracious predators of other insects. They also readily bite when they come into contact with human skin for reasons that are not clear. Due to infrequent encounters, sensitization is rare [12].

True Bugs (Heteroptera)

Members of the conenose subfamily (Triatominae) feed primarily on vertebrate blood, and many of these inject an anesthetic to lessen the pain. Feeding bites usually occur at night, often on soft parts such as lips. For this reason, conenose bugs are also called “kissing bugs.” Within the USA, most human encounters with bites are during camping trips, although indoor bites do occur. In some areas of the western and southwestern USA, bites are common [13]. Allergic reactions from repeat encounters can lead to systemic allergic reactions, and desensitization is effective in preventing recurrence (reviewed by Moffit et al. [14]).

Assassin bugs (Reduviidae) inflict painful bites with their large piercing mouthparts. These bites are given in self-defense. Similarly, aquatic bugs, including the giant water bugs (Belostomatidae) and other predatory aquatic Heteroptera inject neurotoxins into their prey. Bites are given in self-defense, and can be excruciating,

despite the absence of redness and swelling. While the insects are common, bites are infrequent and therefore sensitization is unlikely.

Arachnids and Centipedes

If inspected early, bites of both centipedes and spiders consist of two closely spaced punctures, whereas other arthropods produce a single puncture. Members of these two groups inject venom with a pair of specialized appendages near the mouth (chelicerae in spiders, forcipules in centipedes). The spacing between the chelicerae or forcipules determines the spacing of the skin punctures. Reactions to bites of most of these species (e.g., wolf spiders, Lycosidae) are typically minor, with pain and swelling dissipating within a couple of days. Many spiders will bite when trapped, but widow spiders (Theridiidae: *Latrodectus*) are the most dangerous North American species. Their bites are occasionally followed by systemic neurological reactions to the injected neurotoxin, such as cramping, nausea, and other symptoms. House centipedes and hunting spiders often shelter in shoes or clothes and will readily bite if those clothes are put on, agitating the animal while also trapping it next to the skin.

Though the small bites of brown recluse spiders (Sicariidae: *Loxosceles reclusa*) and other recluse species are often painless, their venom can lead to mild, teardrop-shaped papules of underlying necrosis. These lesions usually heal without incident. Recluse spiders rarely bite people, and they are falsely blamed for many other unrelated skin problems. Of note, the species of recluse spider are not distinguishable without dissection. The medical literature has numerous case reports that attribute unusual reactions to spider bites in general and recluse bites in particular; rarely is a specimen available to confirm the association. Of these, rare AGEP-like reactions associated with recluse bites may be true [15].

Larval chiggers (Trombiculidae: *Trombicula*) have evolved to feed on snakes. When they contact with humans, they crawl over the skin before they settle into constricted areas (e.g., armpits, groins, beneath clothing elastic, and the backsides of knees) and there attempt to feed. The tiny mites (less than 0.2 mm) inject saliva that breaks down skin cells and forms a hard feeding tube called a stylostome, through which they drink liquefied tissues for several hours before dropping off. The chiggers are easily brushed off but the stylostome remains. The immune reaction to the stylostome results in an intensely pruritic papule that peaks in intensity within days but may persist for several weeks, with peak incidence over the summer. Reactions in the genitalia can result in functional impairment [16]. These immunological reactions grow more intense with each exposure [17]. It is a popular misconception that the mite remains in the lesion.

Other mites that can be problematic for people include those that parasitize home-invading animals such as birds and rodents. Bites occur within the home near the nesting site of the rodent or bird. Outdoor populations of predatory itch mites (e.g., *Pyemotes tritici* and the newly invasive *P. herfsi*) can also cause significant reactions where outbreaks occur [18].

Ticks

Ticks generally feed without being noticed. Repeat exposure and the development of sensitization may protect against infection by tick-borne diseases by prompting the person to remove the tick early. Secondary infection at the bite can occur if mouthparts are left in the skin during tick removal. Diseases carried by ticks sometimes produce characteristic rashes, such as spots on hands and feet due to Rocky Mountain spotted fever, a bull's-eye caused by Lyme disease, or a similar-looking bull's-eye symptomatic of southern tick-associated rash illness (STARI). Some tick species can induce a progressive paralysis when feeding, but removal of the tick leads to almost immediate recovery.

Female hard ticks (Ixodidae) lay eggs in one large mass (up to 20,000 eggs), which hatch into a mass of tiny larvae known as "seed ticks." The large number of bite reactions can be distressing to those that have been sensitized.

Rappo et al. [19] describe a large case series of anaphylaxis from tick bites (34 cases) presenting to an Australian ER over a 2-year period. This is notably different from what is typically encountered in US emergency departments, with one case series identifying tick as the trigger in two cases over a 1-year period [20] and tick rarely being reported as a trigger for anaphylaxis otherwise.

Large local reactions are more commonly reported with tick bites, and recently the sensitization to alpha-gal from tick bites has been implicated in cross-reactive anaphylactic reactions to red meat [21].

Bedbugs

Bedbugs (Heteroptera: Cimicidae: *Cimex lectularius*) are common pests in urban environments. Sensitization follows the pattern of other biting insects where exposure is intense, such as mosquitoes. These bugs feed at night on skin not covered by clothing. The recurrent biting of a single bug can result in a classic row or cluster of bites. The reddish fluid that they excrete can be seen on bedding and clothing, and asking a patient about the presence of these flecks of blood on the sheets can help uncover the cause of lesions. It can be difficult to locate the bugs, which are very small and hide in mattresses or other nearby locations during the day. The literature on bedbug reactions is reviewed by Reinhardt [22].

Fleas

Fleas (Siphonaptera) vary with regard to the length of time they attach to feed. The majority of fleas that bite humans, dogs, and cats are cat fleas (Pulicidae: *Ctenocephalides felis*). As with most fleas that bite humans, this species is incapable

of completing its life cycle on human blood, and attempts to feed are brief but potentially persistent. Reactions similar to “Skeeter Syndrome” are common into adulthood with cat fleas. Other species infrequently encountered can remain attached for days. Because these encounters are rare, sensitization is unlikely [23].

Lice

Head lice (Pediculidae: *Pediculus humanus capitis*) can cause sensitization after prolonged or recurrent infestations. Rarely this can result in systemic symptoms [24]. Intensive screening in US schools makes this sensitization less common. Infestations are suggested by finding nits (eggs) cemented onto hair shafts within 1 cm of the scalp, primarily above the ears to the back of the head. Past infestations are suggested by finding light-colored (empty) nits attached farther than 15 mm from the scalp. Body lice cement eggs to clothing, and populations that infrequently change clothing can develop intense exposure and sensitization.

Pubic lice (Pthiridae: *Pthirus pubis*) and their nits, though typically restricted to the pubic area, can be found on head and facial hair, including the eyelashes when infesting children. Two nits per follicle are not unusual for this species. Small blue spots and itching may be seen at the site of feeding. A variety of rashes, including id reactions, are associated with louse infestation, reviewed by Ko and Elston [25].

Scabies

Human scabies mites (Sarcoptidae: *Sarcoptes scabiei hominis*) create short (5–15 mm), straight, or curved burrows just under the skin, typically with a small raised area at the end where the mite resides. This sign is frequently obscured by scratching. Common sites include the webs of the fingers and groin. Dog scabies mites (*S. scabiei canis*) can cause itching in humans where skin contact is made with an infested animal, but these mites cannot maintain an infestation in humans who have an intact immune system. Allergic reactions are to a number of antigens in the mites and their feces retained in the mite burrows. Crusted scabies is a more debilitating immunologic response in which great numbers of mites are present. In these patients, there is greater serum IgE and IgG4 binding to mite apolipoprotein than in those with ordinary scabies [26].

Allergic Immune Responses

Any arthropod that repeatedly feeds on humans is capable of generating allergic sensitization. Experimental studies in humans and other animals have shown that there is a predictable sequence of T-cell and IgE-mediated responses that can

Table 13.3 Implications of allergic inflammation after arthropod bites or stings

Stage of allergic sensitization	Allergic inflammation	IgE activity	T-cell activity	Implication
1	None; some species may induce mast cells directly producing transient itch	Absent	Absent	No previous contact
2	Delayed-onset papular urticaria lasting days	Absent	Present	Few previous encounters
3	Immediate wheal and flare proceeding into papular urticaria	Present	Present	Multiple previous encounters
4	Immediate wheal and flare that resolves within hours	Present	Absent	Many previous encounters
5	None; possibly transient itch during feed	Absent or very low	Absent	Very many previous encounters

provide clues as to the level of exposure that a person has endured with a given species. These stages of sensitization were outlined by Mellanby in 1946 [27] and further described by Feingold et al. [28], as beginning with a period of no allergic inflammation, referred to as stage 1 (see Table 13.3). During stage 1, only innate reactions, if any (as described above), are present. Even insects considered to produce characteristically pruritic lesions induce no symptoms on initial exposure [23]. As sensitization proceeds, the allergic inflammation becomes more alarming to patients, and these diagnostically useful characteristics are obscured. Contrary to popular belief, the degree of allergic inflammation itself is not diagnostically useful, as this progression of stages is similar in bedbugs [29], fleas [30], sandflies [31], and mosquitoes [27].

Because progression through these stages is dependent upon the frequency and duration of exposure, family members often have different reactions to bites [32]. Blood-feeding arthropods that are adapted to feeding on humans often produce anesthetics and have behavioral adaptations that prevent detection while feeding. Mosquitoes are the most commonly encountered blood-feeding insects in North America, although fleas, lice, and other species can be common in certain environments. With frequent exposure to a particular species (e.g., anthropophilic mosquitoes), infants are typically at stage 1 (no sensitization), toddlers at stage 2 (delayed sensitivity), school-aged children at stage 3 (delayed and immediate sensitivity), adolescents at stage 4 (immediate sensitivity only), and adults at stage 4 or 5 (desensitization). When there is less intense exposure, the age ranges are shifted. When a person moves to a new area and is bitten by a new species of mosquito, their reactions to that new species will likely go through these stages beginning at stage 1 [33]. The stages are specific to a species (in some cases to the genus) of arthropod that is biting, so that a person may be at stage 5 for a particular species of mosquito, stage 3 for another species of mosquito, stage 2 for a species of tick, and stage 1 for species by which he has not yet been bitten. After a period of bite avoidance (such as during winter), inflammation with exposure will be reduced, although when bites resume, the severity of reactions will increase.

Delayed-Type Hypersensitivity Reactions

Papular urticaria refers to delayed-type hypersensitivity to insect bites, and during stage 2 sensitization is a diagnostic challenge to clinicians due to the significant length of time between the bite and the reaction. Because this T-cell sensitization indicates repeated exposure, the culprits of stage 2 sensitization in children are likely to be a common species, such as a mosquito entering homes undetected at dusk. The cause of stage 2 sensitization for an adult is likely to be a less common species (e.g., floodwater mosquitoes) or one that has recently moved into an area (e.g., bedbugs). The inability to definitively identify the cause of papular urticaria generates considerable distress in patients that can lead to illusions of parasitosis. Preconceived notions of a "typical" mosquito bite and the heterogeneity of reactions within a family can impair acceptance of common species as the cause of papular urticaria [34].

The delayed-type hypersensitivity reactions in some individuals are unusually robust and may be accompanied by low-grade fevers and fatigue, with lesions that last a week or more before resolution. Simons and Peng [35] coined the term "Skeeter Syndrome" to refer to patients with such significant delayed-type hypersensitivity reactions to mosquitoes, a diagnosis that is especially well-received with patients. Such pronounced delayed-type hypersensitivity reactions can also occur with fleas [36], bedbugs [37], kissing bugs [38], and other arthropod species that are abundant, particularly during stage 3 reactions.

Insect-induced delayed-type reactions typically occur in patches on exposed skin (characteristically occurring in lines for bedbugs). In contrast, attacks by immature, microscopic mites and ticks can result in hundreds of bites, primarily located at sites of constricted clothing. Exposure to seed ticks and chiggers are the most concerning to patients due to the large numbers of biting arthropods encountered. Chiggers are the immature, parasitic stage of trombiculid mites. T cell reactions to chigger saliva occur on the first exposure due to the formation of a feeding tube (stylostome) within the tissues that takes about a week to break down. The development of localized, immediate-type hypersensitivity reactions to chiggers may occur with repeated exposure to chigger saliva and can be used to locate the microscopic mites before the induction of a stylostome (G. Strickland, personal communication).

Blistering reactions may form when superficial epithelial integrity is retained while intraepithelial connections are severed, as when cantharidin from blister beetles provokes lysis of desmosomes in the stratum spinosum [39]. Vesicles or blisters developing 12–48 h after exposure generally suggest a cytotoxic T-cell response from recurrent antigen stimulation, although the cloudy, pustular response to solenopsin in fire ant venom may be the result of effects on accumulating neutrophils [40]. While the number of antigen-specific cytotoxic T cells are generally insufficient to induce significant blisters following insect bites or stings, situations in which abnormally increased numbers of T cells are circulating in the body are

associated with blistering reactions. Blistering in response to insect bites or stings can occur in the context of lymphoma [41] or recent anaphylaxis [42].

Immediate-Type Hypersensitivity

The excoriations seen in delayed-type hypersensitivity reactions may be an adaptive reflex aimed at physically removing skin that has been infiltrated with helminths deposited along with the arthropod saliva. Delayed-type itch begins prior to the time that most helminths disseminate from the site of inoculation [43]. Preventing the transfer of pathogens at the time of the bite via the induction of immediate itch would seem to be a more effective manner of preventing infection. The appearance of delayed-type hypersensitivity well before the development of immediate-type hypersensitivity for a wide range of biting arthropods suggests that this gap is an important immunologic phenomenon. Mechanistically it may be explained by the activation of B cells and T cells in lymph nodes via different mechanisms. The antigens activating T cells are actively transported to lymph nodes by dendritic cells that were themselves activated at the site of tissue injury. Antigens are presented both via MHC class I and MHC class II molecules so that both CD8 T cells and CD4 T cells are activated. In contrast, the antigens that activate B cells passively flow to lymph nodes. It is interesting to note that stings from social Hymenoptera are a common cause of anaphylaxis, and this venom contains vasoactive amines that may be important in disturbing the normal flow of antigens through tissues [44]. The importance of this delay may be in the danger of an overly aggressive IgE-mediated reaction as seen in anaphylaxis, and T-cell sensitization may be the key to understanding the failure of the immune system to localize allergic inflammation seen in such systemic reactions. Further research into the immunological reactions to mosquitoes and fleas may provide insight into what goes wrong during life-threatening allergic reactions.

Summary

Reactions to arthropod bites are caused by a number of mechanisms, including damage done directly by mouthparts to the skin, the effects of salivary components, and the immune response to those salivary components. Identifying the culprit arthropod after a bite is difficult, but determining the amount of pain caused by the mouthparts at the time of the bite can help determine the size and nature of those mouthparts as one important clue. Likewise, looking for the effects of venom can be helpful, such as experiencing pain out of proportion to actual tissue damage for neurotoxins or bleeding in the case of anticoagulants. Assessing for delayed-type and immediate-type hypersensitivity can provide clues to the number of previous encounters experienced.

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Chapter 14
Arthropods and Human Disease

Michael O'Connell

Introduction

The English word arthropod comes from the Greek words *arthron* and *podos* which when combined means "jointed foot" [1]. Arthropods are invertebrate members of the phylum *Arthropoda* with segmented bodies, jointed limbs, and exoskeletons (cuticles) consisting of a biopolymer of glucosamine called chitin and often mineralized with calcium carbonate [2]. Arguably, arthropods are the most successful animals on the planet as they are present in land, sea, and air and make up over three-fourths of all currently known living animal species, estimated as up to ten million species in total [3]. This diverse group includes spiders, insects, and crustaceans as varied as the smallest mosquito to the Japanese spider crab [4]. Fleas, ticks, lice, flies, and mosquitos are the typical vectors associated with transmission of important human pathogens including bacteria, viruses, *Rickettsia*, parasitic worms, and protozoa. Human disease is most prevalent in developing countries as the endemic zones occur primarily in subtropical and tropical countries—which is typically the ideal environment for the various pathogens and the arthropods that harbor them. However, the geographic distribution of arthropod-borne disease endemic zones is expanding due to numerous factors including rising global temperatures associated with climate change, increased urbanization, and unprecedented ease of global travel due to modern aviation [5]. As a result, arthropod-borne diseases are spread when humans travel between infected and noninfected areas or when infected arthropods are accidentally transported from an endemic to a non-endemic zone.

M. O'Connell, M.D., F.A.A.A.A.I., F.C.C.P.
Division of Pulmonology, Allergy, Critical Care, and Sleep Medicine, Department of Medicine, Ohio State University College of Medicine, 2012 Kenny Road, Room 220, Columbus, OH 43221, USA
e-mail: mocmd@aol.com

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Other social stresses such as poverty, war, and overpopulation may cause a breakdown in a country's public health systems leading to conditions that enhance breeding grounds for vectors such as mosquitos and the neglect of effective control measures. Arthropod-borne human diseases causing significant global impact and concern include malaria, yellow fever, dengue, Chagas disease, chikungunya, and most recently Zika which exploded in Latin America in 2015 and has been associated with a severe developmental abnormality, microcephaly, in children born to Zika-infected mothers in Brazil [6–8]. These arthropod-borne diseases can result in a highly diverse range of clinical presentations including being asymptomatic, suffering mild flu-like symptoms, to severe morbidity and death depending on the pathogen involved and the age and health status of the infected human host.

Arthropod-Borne Disease Characteristics and Transmission

Human contact with various arthropods can result in bites, stings, and allergic reactions. Stings and allergic reactions are covered elsewhere in this textbook in extensive detail. Arthropod bites can result in both direct skin injury and the transmission of arthropod-borne disease. Mechanisms of skin injury include [9]:

- Mechanical trauma
- Injection of irritant, cytotoxic, and/or pharmacologically active substances
- Injection of allergenic substances
- Secondary infection
- Invasion of host tissues (e.g., myiasis with fly larvae)
- Contact reactions
- Reactions to retained mouthparts

Humans contract arthropod-borne diseases when pathogens are transmitted from their natural hosts (reservoirs) to humans via the arthropod vector. As noted previously, the most common arthropod vectors are mosquitos, flies, fleas, ticks, and lice. Transmission occurs either via mechanical (sometimes referred to as passive) or biological (active) means [10]. During mechanical transmission, the arthropod vector deposits pathogens onto a surface from which a host either absorbs or ingests the organisms. For example, flies can carry bacteria or other disease-causing organisms on their mouthparts and feet obtained from infected human feces. If a human subsequently eats food that has been contaminated via this process, dysentery or other diarrheal disease may occur.

Pathogens can be passed to humans by vectors utilizing biological transmission via several distinct mechanisms [10]:

- Inoculation
- Regurgitation
- Defecation
- Crushing of vector

Transmission by inoculation occurs when an arthropod vector injects saliva containing the pathogen into the host while it feeds. *Anopheles* mosquito transmission of malaria parasites are a classic example of biological transmission via inoculation [11]. Transmission by regurgitation occurs when the vector vomits the pathogen into the host while it feeds. Fleas transmit bubonic plague using this mechanism [12]. The etiologic agent of plague, *Yersinia pestis*, multiplies rapidly in the gut of the flea causing a blockage. When the flea attempts to feed on a host, it cannot fully ingest the host's blood, and *Yersinia* bacteria is subsequently regurgitated into the host. Transmission by defecation occurs when the vector defecates into a wound in the human skin, and subsequent mechanical trauma (rubbing and scratching of the wound) by the host results in entry of the pathogen into the body. The transmission of Chagas disease (North American sleeping sickness) occurs by this mechanism [13]. Kissing bugs (*Triatoma* sp.) bite humans leaving a wound in the skin which they subsequently defecate into. Lastly, human transmission via crushing of the arthropod vector occurs when a human crushes an arthropod on the skin and rubs the pathogen into the skin in an attempt to remove the remnants of the insect from the skin surface. The body louse can transmit epidemic typhus in this manner [14].

Arthropod-Borne Diseases in Humans

Table 14.1 lists the common arthropod-borne diseases in humans by arthropod vector class along with the etiologic agent and method of human transmission. Selected arthropod-borne diseases are discussed in more detail below:

Class Arachnida

Tick-Borne Illnesses

Babesiosis

The disease is named after the genus of the causative protozoan parasitic organisms, *Babesia*, and the disease is transmitted by the bite of *Ixodes scapularis* ticks (deer ticks) [15]. Babesiosis is considered an emerging disease in the USA and Europe, and it occurs in the USA primarily in the Northeastern and Midwestern regions during the warm summer months with *B. microti* being the predominant causative organism. The clinical presentation resembles malaria with onset of fever, malaise, sweats, shaking chills, and hemolytic anemia occurring within 1–4 weeks after a tick bite [16]. Diagnosis can be confirmed by examination of a Giemsa-stained thin blood smear and identifying the parasites reproducing in red blood cells where classically four merozoites asexually bud in cross-shaped inclusions that appear similar to a Maltese cross. Serologic and polymerase chain reaction (PCR) tests are also

Table 14.1 Common arthropod-borne disease vectors

Arthropod vector	Human disease	Etiologic agent	Method of human exposure
Class Arachnida			
Mite: *Leptotrombidium* sp. (red mites)	Scrub typhus (Tsutsugamushi disease)	*Rickettsia tsutsugamushi* (bacteria, intracellular)	Larval mite bite
Mite: *Liponyssiodes sanguineus* (mouse mite)	Rickettsialpox	*Rickettsia akari* (bacteria)	Larval mite bite
Tick: *Dermacentor* sp.	Tularemia	*Francisella tularensis* (Gram-negative bacteria)	Tick bite
Tick: *Dermacentor* sp. and other ixodid ticks	Rocky Mountain spotted fever	*Rickettsia rickettsii* (bacteria)	Tick bite
Tick: *Ornithodoros* sp.	Endemic relapsing fever	*Borrelia* sp. (bacteria, spiral shaped)	Tick bite
Tick: *Ixodes* sp.	Babesiosis	*Babesia microti* (parasite: protozoan)	Tick bite
Tick: *Ixodes* sp.	Lyme disease	*Borrelia burgdorferi* (bacteria, spiral shaped)	Tick bite
Tick: *Dermacentor variabilis*, *Amyblyomma americanum*	Ehrlichiosis, Sennetsu fever	*Ehrlichia canis*	Tick bite
		E. sennetsu	
		E. chaffeensis	
		E. equi	
		E. phagocytophilia (bacteria, intracellular)	
Tick: *Dermacentor* sp.	Colorado tick fever	CTF virus, Eyach virus, or strain S6-14-03 (Reoviridae)	Tick bite
Tick: *Amyblyomma americanum* (Lone star tick)	**No name for disease yet; <10 reported cases**; fever, fatigue, diarrhea, thrombocytopenia, and leukopenia	Heartland virus, a *Phlebovirus*	Tick bite
Tick	Russian spring-summer encephalitis, louping ill encephalitis, Langat encephalitis, Powassan encephalitis, Omsk hemorrhagic fever	Russian spring-summer encephalitis, louping ill encephalitis, Langat virus, Powassan virus, Omsk hemorrhagic fever virus (*Flaviviridae*)	Tick bite

Tick	Nairobi sheep fever, Crimean hemorrhagic fever	Nairobi sheep disease virus, Crimean-Congo hemorrhagic fever virus (*Bunyaviridae*)	Tick bite
Tick	Severe fever with thrombocytopenia syndrome (China)	Severe fever with thrombocytopenia syndrome virus (SFTSV, *Phlebovirus*)	Tick bite
Class Crustacea			
Copepod: *Cyclops* sp.	Diphyllobothriasis, fish tapeworm	*Diphyllobothrium latum* (parasite: cestode, tapeworm)	Arthropod is the first intermediate host, and then man swallows infected fish
Copepod: *Cyclops* sp.	Sparganosis	*Diphyllobothrium spirometra* (parasite: cestode, tapeworm)	Human swallows infected *Cyclops*
Copepod: *Cyclops* sp.	Dracunculosis	*Dracunculus medinensis*	Human swallows infected *Cyclops*
Crabs, crayfish: various freshwater species	Paragonimiasis	*Paragonimus westermani*	Human eats infected crustacean.
Class Insecta			
Lice: *Pediculus humanus*	Epidemic typhus	*Rickettsia prowazekii*	Louse bite contaminated by louse feces or crushing louse on skin
Lice: *Pediculus humanus*	Trench fever, bacillary angiomatosis, bacillary peliosis	*Bartonella quintana* (Gram-negative bacteria)	Louse bite contaminated by louse feces or crushing louse on skin
Lice: *Pediculus humanus*	Louse-borne relapsing fever or epidemic relapsing fever	*Borrelia recurrentis* (bacteria, spiral shaped)	Louse bite contaminated by louse feces or crushing louse on skin
Flea: *Xenopsylla cheopis* and various other rodent fleas	Plague	*Yersinia pestis* (Gram-negative rod-shaped bacteria)	Flea bite and feces of flea
Flea: *Xenopsylla cheopis*	Murine typhus	*Rickettsia typhi* (bacteria)	Flea bite and feces of flea

(continued)

Table 14.1 (continued)

Arthropod vector	Human disease	Etiologic agent	Method of human exposure
Flea: *Xenopsylla cheopis* and various other rodent fleas	Rat tapeworm infection	*Hymenolepsis diminuta* (parasite: cestode, tapeworm)	Swallowing infected flea
Flea: various species	Dog tapeworm infection, dipylidiasis	*Diphylidium caninum* (parasite: cestode, tapeworm)	Swallowing infected flea
Bug: *Triatoma* species, (kissing bug, reduviid bug)	Chagas disease	*Trypanosoma cruzi* (parasite: protozoan)	Rubbing infected feces on mucous membranes or the skin
Beetles: flour beetle	*Hymenolepis*	*Hymenolepis nana* (parasite: tapeworm, cestode)	Swallowing infected beetle
Fly, gnat: *Glossina* sp. (tsetse fly)	African trypanosomiasis, African sleeping sickness	*Trypanosoma brucei rhodesiense* and *T.b. gambiense*	Bite of infected fly
Fly, gnat: *Simulium* sp. (black fly)	Onchocerciasis, river blindness	*Onchocerca volvulus* (parasite: roundworm, nematode)	Bite of infected fly
Fly, gnat: *Chrysops* sp.	Tularemia	*Francisella tularensis* (Gram-negative rod-shaped bacteria)	Bite of infected fly
Fly, gnat: *Phlebotomus* sp., *Lutzomyia* sp. (sandflies)	Leishmaniasis	*Leishmania donovani* (visceral, dumdum fever, kala-azar), *L. tropica* (cutaneous, oriental sore, Delphi boil), *L. braziliensis* (mucocutaneous, espundia, American leishmaniasis, chiclero ulcer) (parasite: protozoan)	Bite of infected fly
Fly, gnat: *Phlebotomus* sp. (sandfly in Peru, Ecuador, and Columbia)	Bartonellosis, Oroya fever, Carrion's disease	*Bartonella bacilliformis* (Gram-negative bacteria)	Bite of infected fly
Fly, gnat: *Chrysops* sp. (mango flies)	Loaiasis, eye worm	*Loa loa* (parasite: nematode, roundworm)	Bite of infected fly
Fly, gnat: sandfly	Sandfly fever, Rift Valley fever	Sandfly fever Naples virus, Sandfly fever Sicilian virus, Rift Valley fever virus (*Bunyaviridae*)	Bite of infected fly
Mosquito: *Anopheles* sp.	Malaria	*Plasmodium falciparum*, *P. malariae*, *P. vivax*, *P. ovale* (parasite: protozoan)	Bite of infected mosquito
Mosquito: various species	Bancroftian filariasis, filarial elephantiasis	*Wuchereria bancrofti* (parasite: nematode, roundworm)	Bite of infected mosquito

Mosquito: various species	Malayan filariasis, filarial elephantiasis	*Brugia malayi* (parasite: nematode, roundworm)	Bite of infected mosquito
Mosquito: various species	Dirofilariasis	*Dirofilaria immitis* (parasite: nematode, roundworm)	Bite of infected mosquito
Mosquito: *Aedes aegypti*	Yellow fever	Yellow fever virus (*Flaviviridae*)	Bite of infected mosquito
Mosquito: *Aedes* sp.	Dengue fever, breakbone fever	Dengue fever virus (*Flaviviridae*)	Bite of infected mosquito
Mosquito: *Culiseta melanura*, *Coquillettidia pertubans*, *Aedes vexans*	Eastern equine encephalitis	Eastern equine encephalitis virus (*Togaviridae*)	Bite of infected mosquito
Mosquito: *Aedes triseriatus*	La Crosse encephalitis	La Crosse encephalitis virus (*Bunyaviridae*)	Bite of infected mosquito
Mosquito: *Culex* sp.	Saint Louis encephalitis	Saint Louis encephalitis virus (*Flaviviridae*)	Bite of infected mosquito
Mosquito: *Culex* sp., *Culex tarsalis*	Venezuelan equine encephalitis, western equine encephalitis	Venezuelan equine encephalitis virus, western equine encephalitis virus (Alphaviruses)	Bite of infected mosquito
Mosquito	Chikungunya forest fever	Chikungunya virus, Mayaro fever, Mucambo fever, O'nyong'nyong fever, Pixuna fever, Ross River fever (Alphaviruses)	Bite of infected mosquito
Mosquito	Fevers and encephalitis	West Nile fever, Japanese encephalitis virus, West Nile fever, Zika fever, Wesselsbron fever, Kyasanur forest disease virus (*Flaviviridae*)	Bite of infected mosquito
Mosquito	Fevers and encephalitis	Oropouche virus, Bunyamwera, *Bwamba fever*, Guama fever, Oropouche fever, California encephalitis virus (*Bunyaviridae*)	Bite of infected mosquito
Mosquito	Fevers	Chandipura fever, Piry fever (*Rhabdoviridae*)	Bite of infected mosquito



Fig. 14.1 Lone star tick (*Amyblyomma americanum).* Public domain: http://www.cdc.gov/ncidod/dvbid/images/amblyomma-americanum.jpg. Credit*:* DPDx

available [17]. Babesiosis responds to anti-infective therapy, and the current recommendation for first-line treatment is a combination of atovaquone and azithromycin for a period of 7–10 days [18].

Ehrlichiosis

This disease is also named after the genus of the main causative organisms, in this case obligate intracellular bacteria in the family *Rickettsiaceae* which infect and kill human blood leukocytes and monocytes [19]. Deer ticks (*Ixodes scapularis*), American dog ticks, and the lone star tick (*Amblyomma americanum*) [Fig. 14.1] can transmit the infection with the lone star tick being the primary vector with a geographic distribution in the USA involving South Central and Southeastern states [20]. Symptoms usually begin 7–14 days after the bite of an infected tick, and the clinical presentation is onset of flu-like symptoms with headache, fever, malaise, and muscle aches. A petechial rash may occur rarely which resembles the skin rash associated with another rickettsial disease, Rocky Mountain spotted fever [20, 21]. Diagnosis can be made by a PCR assay early in the disease and confirmed by the gold standard immunofluorescence assay (IFA) serologic test with paired samples 2–4 weeks apart showing a fourfold rise in serum antibody [20, 21]. Ehrlichiosis responds to anti-infective therapy, and the current recommended therapy is doxycycline for 14 days [20, 21].

Lyme Disease

Lyme disease derives its name from a cluster of index cases originally thought to be juvenile idiopathic arthritis that occurred in towns in Southeastern Connecticut in 1975, including Lyme and Old Lyme [22, 23]. The primary etiologic agent in

the USA is a bacteria from the *Borrelia* genus called *B. burgdorferi* [23]. Lyme disease is now known to occur throughout temperate climates in the Northern Hemisphere including North America, Europe, and Asia where it is transmitted by infected deer ticks of the *Ixodes* species. In the USA, it is estimated by the CDC that up to 300,000 cases occur annually typically between May and September with the primary geographic distribution being in the Northeast and mid-Atlantic states plus Minnesota and Wisconsin [24]. Lyme disease has a varied clinical presentation and thus can masquerade as other illnesses including various autoimmune and chronic inflammatory diseases since the incubation period after infection can be anywhere from several days to months or even years. Erythema chronicum migrans (ECM), the classic "bull's-eye"-appearing circular rash, appears in 70–80% of patients and represents early localized infection [23]. In the 20–30% of those infected who do not have a skin rash, the disease may manifest as flu-like symptoms followed by various systemic signs and symptoms including acute neurologic complaints such as severe headache, facial palsy, pain syndrome, even presenting as meningitis or encephalitis due to disseminated ECM, and the diagnosis should be considered in anyone who has a history of possible tick exposure in an endemic area [23]. The disease can be confirmed using serologic testing, and the current Centers of Disease Control and Prevention (CDC) protocol recommends performing the enzyme-linked immunosorbent assay (ELISA) first, followed by a confirmatory Western blot test if the ELISA is positive or equivocal. However, the reliability of this testing protocol remains controversial as the two-tiered testing has an overall sensitivity of only 64% in the initial phase of the disease, although this rises to 100% in the subset of people with disseminated symptoms, such as arthritis [25]. Treatment involves oral antibiotics in the early stage of the disease including doxycycline, amoxicillin, or azithromycin. Individuals who present with early disseminated or late infection including symptomatic cardiac disease, refractory Lyme arthritis, or neurologic symptoms consistent with meningitis or encephalitis should be treated with intravenous ceftriaxone [26]. Given the above challenges, prevention measures are highly recommended for humans entering into endemic areas where they can be exposed to infected ticks including wearing hats, long-sleeved shirts, and long pants tucked into boots plus use of insect repellents such as permethrin, *N*,*N*-diethyl-meta-toluamide (DEET), and picaridin [27].

Rocky Mountain Spotted Fever

This disease was originally described in the Rocky Mountain region of the USA, hence the corresponding geographic term in its common name. However, it is now known to be a misnomer as the disease has been found in most states in the continental USA as well as Canada and parts of Central and South America. The etiologic agent of the disease is an obligate intracellular bacteria, *Rickettsia rickettsii*, transmitted by wood ticks of the *Dermacentor* family, principally the American dog tick east of the Mississippi River and the Rocky Mountain wood tick [28]. The disease should be considered in individuals who present with a classic triad of fever, centripetal petechial rash, and a history of a tick bite in the previous 5–7 days [29].

Diagnosis can be confirmed by skin biopsy of the petechial rash and serologic immunofluorescence antibody assays [30]. Treatment consists of a tetracycline antibiotic, typically doxycycline orally continued until the patient has gone 3 days without fever [30].

Tularemia

This disease and the species name of the causative intracellular bacteria take their name from Tulare County in Central California where the disease was first described in ground squirrels in 1911 [31]. The etiologic agent is *Francisella tularensis*, an intracellular Gram-negative coccobacillus [32]. Tularemia is endemic in North America along with parts of Asia and Europe. While the most common mode of transmission occurs via arthropod vectors, tularemia can be spread by direct contact with contaminated animals (particularly rabbits as they serve as reservoir hosts) or ingesting the infected undercooked flesh or contaminated water. Many tick species can spread the causative bacteria including *Amblyomma*, *Dermatcentor*, and *Ixodes* sp. [32]. There are six described clinical presentations of tularemia depending on the site of infection with the most common being ulceroglandular [33]. Most human infections occur within 3–5 days after exposure and the clinical presentation is often severe with high fever and signs of sepsis [32]. Infection can spread to the lymph nodes, and they can suppurate mimicking bubonic plague [32]. Treatment consists of utilization of a regimen including aminoglycoside antibiotics (classically streptomycin or gentamicin) or tetracyclines including doxycycline [34]. Prevention measures for tularemia include following standard barrier and insect repellant regimens to prevent tick bites, wearing rubber gloves when handling rabbits, avoiding ingestion of undercooked wild game rodents, and avoidance of ingestion of water from untreated sources. Of note, the CDC considers *F. tularensis* as a potential biological warfare threat agent as it is easy to aerosolize and highly infective and it has been reported to be part of the bioweapons portfolio of various countries in the past [35].

Class Insecta

Bug-Borne

Chagas Disease

This relatively common disease, also known as American trypanosomiasis and named for the Brazilian epidemiologist who first described it, is caused by the tropical protozoan parasite, *Trypanosoma cruzi*, and transmitted by kissing bugs

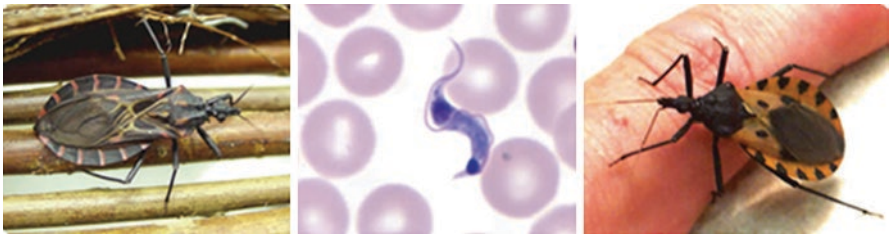


Fig. 14.2 L to R: *Triatoma* kissing bug, *T. cruzi* trypomastigote in a thin blood smear stained with Giemsa, *Triatoma* kissing bug in relationship to human finger. Public domain: http://www.cdc.gov/parasites/chagas/. Credit*:* DPDx

(*Triatoma* sp.) [Fig. 14.2] who bite humans often on the face and then defecate onto the skin in the proximity of the open wound [36, 37]. The WHO estimates that the human prevalence is seven to eight million with affected individuals primarily living in Mexico, Central America, and South America [38]. The clinical presentation occurs in two stages, acute and chronic, with the acute phase occurring shortly after infection but characterized by either an asymptomatic period or relatively nonspecific flu-like symptoms [37, 39]. Local swelling can occur at the site where the parasite entered the body and the most recognized manifestation of acute Chagas disease is Romaña's sign, which is a characteristic unilateral swelling of the eyelid near the site of the bite wound or where the feces of the *Triatoma* sp. were deposited or rubbed into the eye [39]. The symptomatic chronic stage affects the heart, autonomic nervous system, and gastrointestinal tract manifested by dilated cardiomyopathy with rhythm disturbances and megacolon [37, 39]. Treatment is most effective in the acute stage of the disease and typically consists of the antiparasitic agents benznidazole or nifurtimox [37, 39].

Flea-Borne

Murine Typhus

Murine (endemic) typhus occurs throughout the world and is relatively uncommon in the USA with a few cases primarily limited to California and Texas [40]. The etiologic agent is the obligate intracellular bacteria, *Rickettsia typhi*, and the disease is transmitted by fecal contamination of bites from fleas (*Xenopsylla cheopis*) who carry the bacteria from infected rat hosts. Typical presenting symptoms including fever, headache, joint pain, muscle aches, nausea, and vomiting with half of patients developing a discrete rash and/or central neurologic symptoms such as confusion or stupor. The diagnosis can be confirmed by serology if suspected, and treatment consists of tetracycline class antibiotics, typically doxycycline [40].

Plague

The "Black Death" which caused several pandemics in Europe in the Middle Ages is caused by the bacteria, *Yersinia pestis*, and can be transmitted by numerous routes including direct physical contact, airborne transmission, fecal-oral transmission, and arthropod vectors [41, 42]. It is the latter route of transmission that will be focused on here with the bites of infected fleas (*Xenopsylla cheopis)* involved in transmission to human hosts. Fleas infected with *Yersinia pestis* literally starve to death as the rapidly multiplying bacteria form a plug that blocks its stomach and the fleas regurgitate blood infected with the bacteria into the bite wound of the host [41, 42]. Plague is an ancient disease found in archaeologic samples of teeth from the Bronze Age (~3000 BCE), and it has a worldwide distribution [43]. Interestingly, there are several cases yearly in the USA, often in the Southwestern USA and Four Corners region where prairie dogs are the reservoir [44]. There are three main clinical presentations of plague, bubonic, septicemic, and pneumonic, and all three are serious illnesses manifested by high fever, shaking chills, weakness, and abdominal pain with the bubonic form presenting with one or more swollen, tender, and painful lymph nodes typically in the vicinity of the flea bite [41, 42]. Diagnosis can be confirmed by culture of an aspirate from an infected lymph node or blood culture. Several antibiotics are effective in treatment including aminoglycosides and tetracyclines [45].

Fly-Borne

African Sleeping Sickness

Also known as African trypanosomiasis, this arthropod-borne disease is caused by protozoa of the species *Trypanosoma brucei* with the *gambiense* type causing the vast majority of human cases [46]. The disease is endemic in sub-Saharan Africa with potentially 70 million people at risk and causing ~9000 deaths per year as of 2010 [47]. The arthropod vector involved is the tsetse fly (*Glossina* sp.), and the trypanosome is directly transmitted to humans via inoculation [Fig. 14.3] [46].



Fig. 14.3 L and R: *Trypanosoma brucei* ssp. in thin blood smears stained with Giemsa. Center: A close-up of a tsetse fly. Public domain: http://www.cdc.gov/parasites/sleepingsickness/. Credit: DPDx

One to 3 weeks after the bite from an infected fly, there is onset of intermittent fevers, headache, pruritus, and joint pain. Parasite invasion of the lymphatic system causes profound swelling of the lymph nodes, and a classic associated finding is swollen lymph nodes along the back of the neck (Winterbottom's sign) [46]. If left untreated, a second stage begins with central nervous system (CNS) involvement after the parasites cross the blood-brain barrier manifested by confusion, poor coordination, neurologic abnormalities such as numbness and dysesthesias accompanied by disordered sleep-wake cycle which gave this disease its common name [46]. Diagnosis involves demonstrating the presence of the trypanosome on a blood smear or from fluid aspirated from a swollen lymph node, and lumbar puncture can be helpful in diagnosing CNS involvement. Treatment for the initial stage of the disease is pentamidine, and the second stage is typically treated with eflornithine alone or in combination with nifurtimox [48].

Leishmaniasis

Leishmaniasis is a major worldwide public health concern with a prevalence of 12 million people infected in 98 countries in Africa, Asia, Central and South America, and Southern Europe, with two million new cases and up to 50,000 deaths annually [49]. The etiologic agents are protozoan parasites of the genus *Leishmania* which are transmitted via the bites of various female sandflies [50]. Leishmaniasis presents with three varied clinical presentations: cutaneous which presents with skin ulcers; mucocutaneous which is manifested by ulcers of the mouth and nose in addition to those involving the skin; and the visceral form (also known as kala-azar which means "black fever") which begins with skin ulcers followed by fever, anemia, and hepatosplenomegaly [50]. Diagnosis can be confirmed by direct visualization of the amastigotes (called Leishman-Donovan bodies) by spreading buffy coat preparations of peripheral blood or aspirates from skin lesions, bone marrow, or infected lymph nodes on a slide to make a thin smear and applying a Leishman or Giemsa stain [50]. Several therapies are available for both cutaneous and visceral leishmaniasis, and treatment is based on the species of *Leishmania* involved and the geographic location where the disease was acquired [51].

Louse-Borne

Epidemic Typhus

Epidemic typhus is so-called because the disease can cause epidemics during and after wars (e.g., the Plague of Athens during the Peloponnesian War) and disruption resulting from natural disasters [14]. The etiologic agent is the obligate intracellular bacteria, *Rickettsia prowazekii*, and the disease is transmitted by the human body louse (*Pediculus humanus*) either by defecation of the bacteria on the skin or by

mechanical crushing of lice bodies with the bacteria subsequently rubbed into skin disrupted as a result of louse bites [40]. Clinical symptoms occur within 1–2 weeks and include severe headache, cough, a rash which begins on the chest and spreads to the trunk and extremities, severe muscle pain, sustained high fever greater than 102 °F, hypotension, photophobia, and changes in mental status including delirium [40]. Diagnosis can be confirmed by the use of PCR testing on blood or skin biopsy of rash-involved skin [40]. Epidemic typhus can be effectively treated with tetracycline antibiotics or azithromycin [40].

Mosquito-Borne

Malaria

Malaria is the major arthropod-borne disease in the world and one of the most impactful diseases worldwide in terms of morbidity and economic impact [52]. The disease is widespread around the globe in tropical and subtropical regions including large portions of sub-Saharan Africa, Asia, and Latin America affecting 214 million humans worldwide in 2015 resulting in 438,000 deaths, 90% of which occurred in Africa [52]. The etiologic agent is a group of parasitic protozoans belonging to the *Plasmodium* type and the transmitting vector is the female *Anopheles* mosquito [Fig. 14.4] whose bite introduces the parasites contained in the saliva of the mosquito into a person's blood [53]. Symptoms begin typically within 10–15 days and include high fevers, headache, fatigue, and vomiting with more severe cases (often associated with *P. falciparum*) associated with jaundice and dark urine (blackwater fever), seizure, coma, and death [53]. Diagnosis is usually made by microscopic examination of thick and thin blood films which demonstrate the intracellular parasite in erythrocytes or antigen-based rapid diagnostic tests [54]. There are numerous treatment regimens available based on the geographic location where malaria was acquired and the specific parasite involved [55, 56]. Regimens typically combine an artemisinin with either mefloquine or pyrimethamine/sulfadoxine. Resistance has developed to several commonly used anti-malarials such as chloroquine and mefloquine with *P. falciparum* demonstrating the highest level of resistance, which is unfortunate since it is typically responsible for the most severe cases of the disease [55].

Because of the widespread geographic distribution of the *Anopheles* mosquito around the world in tropical and subtropical zones, vigorous preventive measures should be instituted including mosquito control measures such as draining areas of standing water, spraying insecticides, the use of physical barriers such as covering exposed skin with clothing and applying insect repellents on exposed skin, the use of insecticide-coated mosquito netting, and the use of prophylactic medications in travelers who plan to visit endemic areas [57]. Given the impact on global health, development of an effective malaria vaccine is a major public health priority, and several candidates remain under development [58].

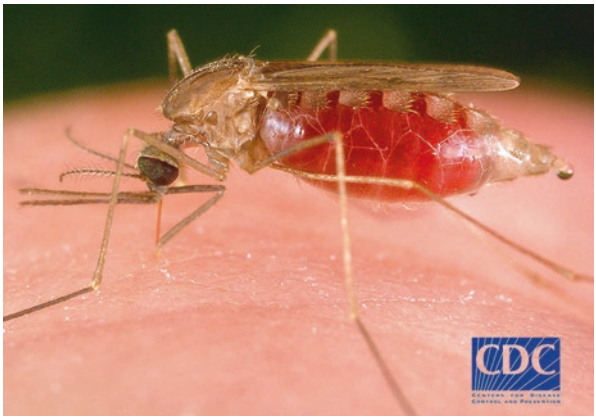


Fig. 14.4 *Anopheles* mosquito feeding on a blood meal. Public domain: http://www.cdc.gov/malaria/about/biology/mosquitoes/freeborni_large.html. Credit: Global Health—Division of Parasitic Diseases and Malaria

Dengue Fever

This arthropod-borne disease, also called "breakbone fever" due to the severe associated muscle and joint pain, is caused by an RNA flavivirus transmitted by *Aedes* mosquitos (typically *Aedes aegypti*) [59]. Dengue is increasing worldwide and is having a major public health impact in tropical and subtropical regions including a recent US outbreak in Hawaii with it being endemic in over 100 countries and responsible for 50 million cases globally each year [60]. The *Aedes aegypti* mosquito [Fig. 14.5] transmits the virus by inoculation and has adapted well to urbanization as it can breed in shallow pools of water, feeds primarily on humans, and often bites during the day in contrast to the *Anopheles* mosquito which carries malaria [59]. Symptoms usually begin between 3 and 14 days after infection and typically include very high fever (can be >104 °F), headache, vomiting, severe muscle and joint pain, and a characteristic skin rash that is described as "measles-like" [61]. For most patients, recovery occurs within 3–7 days after onset of symptoms. In rare cases (<5% of total infected patients), dengue can progress to a critical phase manifested by either dengue shock syndrome or a life-threatening dengue hemorrhagic fever with bleeding and thrombocytopenia [61]. Differential diagnosis can be challenging as dengue resembles clinical syndromes associated with related viruses such as chikungunya and Zika [59, 61]. Diagnosis is typically confirmed by either viral antigen testing (for NS1) or serologic testing [59, 61]. There is no specific antiviral treatment available, and care is typically supportive including maintenance of proper fluid hydration [59, 61]. Preventive mosquito control measures in dengue endemic areas should be implemented including both public health methods including draining of any reservoirs of standing water and spraying using effective insecticides plus personal protective measures including using insect repellents on exposed skin [57]. Mosquito netting is less useful since the *Aedes* mosquito typically bites during the day.



Fig. 14.5 *Aedes aegypti* mosquito feeding on a blood meal. Public domain: http://www.cdc.gov/chikungunya/resources/vector-control.html. Credit: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD)

Yellow Fever

Yellow fever is another arthropod-borne disease caused by an RNA flavivirus transmitted by the bite of an infected female *Aedes* mosquito (typically *Aedes aegypti*) [62]. The disease is common in tropical areas of Africa and South America (where it was spread via the slave trade in the seventeenth century), and there are 200,000 new infections and 30,000 deaths annually with 90% of those occurring in Africa [62]. Typical symptoms include fever chills, nausea, headaches, and muscle pains particularly in the back [63]. While most affected individuals improve within 5 days, some progress with a return of high fever along with abdominal pain and liver dysfunction causing jaundice (the "yellow" in yellow fever) [63]. Diagnosis can be confirmed by either a serologic ELISA test or a PCR test. As with other flaviviruses, there is no specific effective antiviral therapy available, and care is supportive. Preventive measures as described previously for control of the *Aedes* mosquito should be implemented in endemic areas, and there is an effective attenuated live virus vaccine available, which confers protection for 10 years and should be administered to travelers who plan to visit endemic areas [64].

Emerging Encephalitides

West Nile Virus

West Nile virus is another arthropod-borne flavivirus transmitted by various species of mosquitos (particularly *Culex pipiens* in the Northeastern USA), which is an emerging threat and now has spread globally with the first case in the Western Hemisphere occurring in 1999 [65]. The virus is now found throughout the continental USA and parts of Canada, the Caribbean, and Latin America. Birds are the primary reservoir of the virus, and the presence of dead wild birds often predicts a

subsequent outbreak of human disease [65]. Fortunately, the majority of human infections are subclinical and asymptomatic [66]. However, more severe presentations occur including a flu-like West Nile fever syndrome and several neurologic syndromes including meningitis, encephalitis, and a poliomyelitis-like syndrome [66]. Diagnosis can be confirmed by serologic testing of blood or cerebrospinal fluid. Like other flavivirus infections, there is no specific antiviral therapy, so supportive care is the mainstay of treatment [66]. Public health measures to control mosquitos should be implemented as well as instituting personal protective measures in endemic areas [67]. There is no effective vaccine available.

Zika

Zika virus is yet another arthropod-borne flavivirus transmitted by *Aedes* mosquito species, which was named after the Zika forest in Uganda where the virus was first isolated in 1947 [6]. Beginning in 2014, the virus spread to Latin America and has been associated with cases of microcephaly in infants born to mothers infected with Zika in Brazil as well as a link to neurologic abnormalities including Guillain-Barré syndrome in infected adults [8]. In January 2016 the US CDC issued a warning for travelers to endemic areas in Latin America and suggested pregnant women should postpone travel altogether [68]. In early February 2016, the World Health Organization (WHO) declared Zika to be a global public health emergency requiring a coordinated response [69]. The clinical presentation of Zika resembles a mild form of dengue fever with mild headaches, fever, malaise, joint pains, conjunctivitis, and a diffuse maculopapular rash as common presenting complaints [6]. There is no effective treatment, and no prophylactic vaccine is available, so preventive measures described previously and below in more detail including personal protective measures and implementation of public health practices to control the *Aedes* mosquito are essential to reduce the spread of Zika [6, 68].

Prevention and Control

The best ways to prevent arthropod-borne disease transmission are to implement proper sanitation practices, utilize both physical and chemical personal barrier protection, and eliminate arthropod breeding habitats [57, 70]. Proper disposal of garbage and human waste is a fundamental disease control public health practice [70]. Individuals working and living in tropical climates where arthropod-borne diseases are endemic typically wear less clothing due to hot environmental temperatures leaving a significant amount of body surface exposed. Clothing is an excellent physical barrier, but if wearing long-sleeve shirts and full-length pants is impractical due to heat, insect repellents containing plant-derived essential oils (such as citronella and oil of eucalyptus) or synthetic chemicals (such as DEET or picaridin) should be applied as directed to exposed skin [71]. Use of protective mosquito netting is also an effective prevention measure while sleeping [57]. Measures effective in

eliminating arthropod breeding habitats include proper disposal of garbage, elimination of areas of standing water (mosquito breeding grounds), and control of the local rodent population [70]. In developing countries, even the best sanitation measures can fall short of optimal arthropod control, so prevention efforts may be augmented by instituting chemical measures of environmental spraying using approved pesticides (e.g., malathion) [72].

Alpha-Gal Allergy

Of interest to this audience is the association of red meat allergy with the bite of certain ticks. Originally described in 2009 by the Platts-Mills research group at the University of Virginia [73] and later associated with bites of the lone star tick (*Amblyomma americanum*) [74] and in Australia by van Nunen and colleagues in association with bites of the Australian paralysis tick (*Ixodes holocyclus*) [75], this syndrome is due to formation of IgE antibodies to galactose-alpha-1,3-galactose (alpha-gal) in humans as a result of the tick bite. Subsequent fascinating medical detective work by Platts-Mills and colleagues have demonstrated an association between lone star tick bites and subsequent production of IgE antibodies to alpha-gal resulting in two distinct clinical presentations of anaphylaxis: (1) immediate-onset anaphylaxis during first exposure to intravenous cetuximab and (2) delayed-onset anaphylaxis occurring 3–6 h after ingestion of red meat such as beef and pork [74]. Cases of anaphylaxis to red meat in the USA are geographically distributed in the Southern, Eastern, and Central USA consistent with the distribution of the lone star tick, and additional cases have been described in Europe associated with bites of the European castor bean tick (*Ixodes ricinus*) [76].

Conclusion

Arthropod-borne diseases have historically had significant human impact globally, and their increasing geographic spread and reach in the early twenty-first century due to climate change, urbanization, international travel, and breakdowns in the public health infrastructure is concerning to the WHO and country-level public health authorities in developing countries and the USA. Education of the public to limit high-risk behaviors and comply with travel advisories, use of personal protective measures, and implementation of optimal public health control practices to disrupt the breeding environment for arthropod vectors will increasingly be necessary to control the spread of arthropod-borne disease. Long-term prophylactic solutions to protect the public such as vaccines remain out of reach for common arthropod-borne diseases such as malaria and dengue, but research efforts in the public and private sector are expanding due to the increasing threat to public health.

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Part III

Chapter 15
Behavior, Ecology, Natural History, and Distribution of Stinging Hymenoptera

Justin O. Schmidt

Ouch, something stung me! This, or a similar expression, is a familiar utterance to most people and something often regaled by patients during medical visits. Insect stings are medically important for two reasons. First, they can induce medically important reactions, a topic of immediate concern to healthcare workers and the general public. Second, and perhaps less obvious though important, is the morbidity associated with the sting. This morbidity can be elicited as extreme patient fear and phobia that severely and negatively impacts the patient's and family members' qualities of life [1]. From a clinical perspective, patient fear and apprehension is important and requires addressing. Too often human psychological aspects surrounding stinging insects become de-emphasized in both education and clinical settings relative to the more potentially life-threatening medical reactions. This chapter is designed to bridge the gap between the classic medical and the psychological aspects of insect envenomation by providing information on human behavior toward stinging insects and on the behavior, biology, and natural history of the stinging insects themselves. This information can be used to counsel patients vis-á-vis their fear of, and concerns about, stinging insects and thereby improve patient safety.

Stinging Insect Behavior

Why do insects sting? And how can I prevent being stung? These important questions can be best answered not from a human's perspective, but from the insect's perspective. We need to imagine being the insect and viewing the world through the

J.O. Schmidt, Ph.D.
Southwestern Biological Institute, 1961 W. Brichta Dr, Tucson, AZ 85745, USA

Department of Entomology, University of Arizona, Tucson, AZ, USA
e-mail: ponerine@dakotacom.net

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insect's eyes. If we can do this we are able to understand why insects sting and how we can prevent being stung.

The stinging insect views the world as a place fraught with dangers. Enormous dangers are large ever-hungry predators looking for a meal of tasty insects. One of the ways to deter large predators is to sting them, a defense that often works by stopping the attack and driving away the predator. An important point is that the stinging insect must evaluate the potential risk and act according to that evaluation. If the large animal or human is acting in a way perceived as threatening, even if not, or if unintentionally, the insect responds by attempting to sting. The insect cannot know our intentions: it can only respond to how we act. The stinging insect is not "out to get us"; it is our actions perceived to be threatening that cause the stinging event. For this reason, the personal actions people take are key to preventing or reducing stings and are a factor squarely under our control.

The risk of being stung depends greatly upon the social structure of the stinging insect. If the insect is social, that is, it lives in a colony with other adult workers, a queen (or queens), and young, it is more likely to sting an intruder than if it is a solitary species where each female raises her own young in individual small nests. All of the medically important stinging insects are social, including honey bees (*Apis*), yellowjackets (simply called "wasps" in Europe and much of the world) of the genera *Vespula* and *Dolichovespula*, the bald-faced hornet ("white-faced hornet," *Dolichovespula maculata*), the aerial yellowjacket ("yellow hornet," *Dolichovespula arenaria*), hornets (*Vespa* and *Provespa*), ants, and bumble bees (*Bombus*). For in-depth taxonomic and entomological analyses of these species, see Jacobson [2]. These species are medically important because they are the ones that most frequently sting people. And they sting people more frequently because they have much more to lose—nestmates, young, reproductives, and food reserves—than solitary species which have little to lose, mainly themselves [3]. A human analogy to the social versus solitary insect situation is a parent pushing a baby in a carriage on a sidewalk, versus walking alone, and being attacked by a robber. In the first case the person is likely to fight back and in the second to flee.

Social stinging insects experience two dramatically different environments during their daily lives. These are environments away from the home nest while foraging for food, water, or resources, and environments in or near the nest. When away from the nest, the insect has nothing to defend other than itself. For this reason when it encounters a person or predator, it mainly wants to survive, usually by escaping rather than by fighting (stinging). If actually grasped, restrained, or held, it will sting in personal defense; otherwise, it will not sting. This behavior is well known to entomologists who sometimes for show knock a bee off a flower or flick a yellowjacket off a scrap of meat and the insect simply buzzes off. This is not generally recommended for the general public (the insect might get caught in hair and sting), but woe to anyone who steps barefoot on a bee on a clover flower.

In contrast to their docile behavior of away from their nest, social insects near their nest are highly defensive. Here they have not simply themselves to defend, but much more, their young and nestmates. When a person intrudes near the nest, some of its inhabitants rush out in defense of the nest, often ready to sting. Their whole

reproductive potential is at stake. Thus, they are willing to risk it all, that is, their lives in the process of attempting to sting. This makes sense in the context that most worker social insects are reproductively sterile and pass their genes on only by helping their reproductive mothers or kin [4].

Human Behavior

Over thousands of generations, humans and our primordial ancestors developed a strong genetic fear and aversion to animals that directly attacked them, including snakes, spiders, and insects, and instinctively learned to avoid them. This instinct is biologically rooted [5, 6]. Those individuals who lacked a fear of attacking animals, large or small, or who failed at avoiding them were frequently attacked or envenomated, with dire consequences, including death. Genes governing detection and fear of dangerous animals were positively adaptive for individuals possessing those genes. Those individuals with genes that did not confer strong detection and avoidance abilities were slowly eliminated from the gene pool. This evolutionary picture explains much of modern human behavior toward stinging insects. When a bee or wasp buzzes a person, a typical response is to flail or swat at the insect in hopes of driving it away. Curiously, ordinary flies with somewhat similar buzzing flight often elicit the same automatic human behavior.

In the evolutionary race between stinging insects and mankind, one could argue that the stinging insects are winning. Evidence comes in the form of our oversized fear of stinging insects, our emotional reactions both verbally and physically to them, our overexuberance in trying to kill them, and our sometimes overly emotional seeking of medical solutions. The insects won in the respect that we fear them and leave them alone, exactly what is in their best interest. We lost because our fear and reactions sometimes operate against our own best interests. For example, our irrational fear of bald-faced hornets often leads us to kill colonies, yet bald-faced hornets and yellowjackets are our friends who prey on countless numbers of flies that spread disease and torment our livestock [7].

How can we modify our innate fear and dislike of stinging insects to improve our quality of life? No magic bullet exists, but we can improve our attitude in several ways. We can take lessons from medical approaches to arachnophobia. One of the keys to eliminating arachnophobia is to get the patient to look at distant images or cartoons of spiders, working ever harder to appreciate that spiders can have their own intrinsic beauty [8]. As the patient becomes less fearful, progressively more challenging steps often can be taken, such as holding a picture of a spider. Ultimately many people can completely overcome their fear and even hold a tarantula. A similar approach can be used with people phobic of stinging insects. Most people find butterflies, giant silk moths, and gem-colored beetles to be beautiful. Many stinging insects are equally beautiful, albeit smaller. Bumble bees are cute round, colorful, fuzzy balls on wings. Yellowjackets appear to be beautiful yellow and black flying paintings. Some wasps are iridescent green, blue, or purple, sometimes with flashy

Table 15.1 Death rates in the USA from various causes

Cause	Number of deaths/year	Death rate per 1,000,000/year	% of total deaths	Reference
All causes	2,421,843	8373	100	[a]
Heart disease	681,793	2357	28.1	[a]
Cancer	595,495	2058	24.6	[a]
Smoking	443,595	1534	18.3	[c]
Diabetes	69,656	241	2.7	[a]
Motor vehicle accidents	45,793	158	1.9	[a]
Suicide	31,335	108	1.3	[b]
Homicide	17,923	62.0	0.74	[b]
Asthma	3785	13.1	0.15	[b]
Drowning	3439	11.9	0.14	[b]
Slips and falls while walking	619	2.14	0.025	[b]
Hot weather	392	1.35	0.016	[b]
Lightning	52	0.18	0.0021	[b]
Lawn mowers	48	0.17	0.0020	[b]
Insect stings (all)	60	0.21	0.0025	[b]

[a]CDC, Leading causes of death, US 1999–2006; http://webappa.cdc.gov/sasweb/ncipc/leadcaus10.html
[b]CDC Wonder Database; http://wonder.cdc.gov/cmf-icd10.html
[c]Anon 2009. Smoking-attributable mortality, years of potential life lost, and productivity losses – United States, 2000–2004. *JAMA* 301: 593–94

orange wings. When a person takes the opportunity to see the beauty in many stinging insects, fear naturally decreases. This can be followed by learning about the wondrous lives and behaviors of these amazing insects. Honey bees and bumble bees provide excellent examples, but paper wasps and yellowjackets also do.

Another way to reduce fear of stinging insects is education about the real risks of dying "upon the next sting." Are the real killers in our lives allergic reactions to stinging insects? No, the real killers are heart disease, cancer, smoking, diabetes, and an assortment of behaviorally related activities including suicide, homicide, and drowning (Table 15.1). In comparison, insect stings kill only very few people, in the same order of magnitude as lightning and lawn mowers and many times fewer than slipping while walking or hot weather. Many of the major causes of death can be reduced by changes in behavior including eating a diet containing less fat and high animal protein, not smoking, and by exercising more. Perhaps through learning to prevent insect stings and proper medical treatment, the number of sting deaths can also be reduced.

For those estimated 1–4% of the populace who are allergic to insect stings, some hope can be offered [1]. If an allergy rate of 2% is assumed and the US population is 320 million, then 6.4 million people are allergic to stings. This translates into a 1 in 100,000 chance of death yearly for a sting-allergic person. The take-home message is: fear less, don't overreact and ruin your quality of life, but be sensible and take precautions.

Ecology and Distributions

Honey Bees

Honey bees are the most familiar and beloved insects worldwide. The common honey bee known to Europeans, Africans, North and South Americans, and much of the rest of the world is but one species in the genus *Apis*, a collection of nine commonly recognized species plus numerous subspecies, or races, within these species. Most of these, including the giant honey bees, the dwarf honey bees, and the Eastern hive bees, are found in southern or eastern Asia. Giant and dwarf honey bees make nests consisting of a single comb that hangs in the open. The common honey bee (*Apis mellifera*), sometimes called the Western hive bee, and Eastern hive bee (*A. cerana*) usually live in cavities, often in hollows in trees. *Apis dorsata*, one of the two giant honey bees, is famous for its impressive defensive attacks against predators. They build their single comb as large as 1.5 m in length and 0.9 m downward under high branches of tall trees. As many as 156 colonies will aggregate in a single tree or adjoining trees. When threatened, giant honey bees, unlike the cavity-nesting bees that must exit through a restricting hive entrance, can simply fall off the comb and instantly mount immense stinging attacks. With an average population of 15,000–40,000 bees per colony, that makes a lot of bees on the attack. If other nearby colonies are disturbed that attack can be multiplied by a large number, making a truly dangerous situation irrespective of potential allergy problems. In contrast, dwarf honey bees live in small colonies, usually hidden among dense vegetation, and are docile, rarely stinging [9].

Nearly everybody has heard of "killer bees," officially referred to as Africanized bees. Killer bees are simply common honey bees that have an "attitude." They do not like potential predators or intruders and exhibit their displeasure through massive stinging attacks. Perhaps somewhat surprisingly, killer bees are not larger, or necessarily blacker, than the familiar domestic European bees kept in beekeepers' hive boxes; in fact, they tend to be smaller. What they lack in size they make up in behavior. The venom of all species is nearly identical and are apparently completely allergically cross-reactive [10, 11].

Hives of the common honey bee tend to be located within secure cavities of trees, building walls, or abandoned/little used structures. Africanized bees throughout warmer Latin America and approximately the southern third of the USA are less fussy in hive locations, accepting most any cavity in trees, building walls, old boxes, abandoned vehicles, tubs and barrels, abandoned mines, rock cavities in outcrops and stream banks, and even nesting with exposed combs in dense vegetation lacking a cavity. These Africanized bee colonies can be especially dangerous as people tend to intrude unintentionally into the bees' nest area and precipitate an attack.

Prevention of bee stings is important, especially for hypersensitive people when they are outside. Honey bees are a perennial species, and they can be active any time of year, especially when temperature reaches or exceeds 10 °C (50 °F). If hives are disturbed, even in winter, they can mount a stinging attack. Alertness and awareness

are key factors in preventing stings. Eyes and ears are our early warning defenses. When outside, especially in backyards, one should scan for insect flight, paying close attention to insects that "bee-line" in straight flights. These flights suggest bees (or yellowjackets) coming and going from a hive entrance. When using ladders or lawn mowers, first walk and case out the areas, looking for bee (or wasp) nests. If a hive is present, obtain help from a professional exterminator or beekeeper. Pet dogs can be a problem. Dogs tend to snap at flies and bees and their actions can arouse a defensive attack from a nearby unnoticed bee hive. If this happens, all people need to go inside, call the dog and partially open the door to let in the dog, and call an exterminator.

Sensible precautions when around bees usually prevent stings. If a hive location is known, stay clear of the inward and outward flight path to prevent bees from bumping into a person or getting entangled in hair. If buzzed by a bee, freeze all movement and hold breadth while looking for a potential nearby hive entrance and waiting for the bee to leave. Breath is the single most powerful stimulus eliciting a bee attack [12]. NEVER swat at a bee, as motion is the second most powerful stimulates for a bee attack. If actually stung, all people in the area should immediately run into a building or vehicle and keep doors and windows closed.

Honey bees away from a hive present low risk and rarely sting. The biggest risk is a bee becoming entangled in hair or being inadvertently stepped or sat upon. Areas requiring most vigilance are around dense patches of flowers and outdoor picnics. Bees and yellowjackets land on sweets or crawl into soft drink containers and can be inadvertently consumed. A less familiar cause of stings is open vehicle windows. A bee or other stinging insect can fly or bounce in, ending up under lose clothes and stinging. The author once was riding a bicycle and breathing hard through open mouth, and a bee flew in and stung his tongue. No set of precautions is fail-safe; all that can be done is attempt to minimize risks.

Yellowjackets

Yellowjackets typically have an annual life cycle and, consequently, present a stinging risk mainly during late spring until the fall [2]. These warm weather times are when colonies are most populous and most defensive. During winter and spring, yellowjackets exist as single queens hibernating or initiating their colonies and are unlikely to be encountered or to sting.

Like honey bees, yellowjackets present stinging risks in two strikingly different situations: near the nest and away from the nest while foraging for food or materials. The more serious situation is near the nest where yellowjackets will fly out and sting in nest defense. Colony populations are smaller than those of honey bees, and nests are often unnoticed until they attack. Most yellowjacket species nest underground where their nests are hidden from view. Aerial yellowjackets and the bald-faced hornet are exceptions in that they make their nests above ground (or rarely touching the ground) attached to vegetation or on the sides of houses and other man-made

structures. Again, as with honey bees, eyes and ears are our early warning systems. Yellowjacket nests tend to be at the edges where lawns meet shrubbery or walls and along the banks of streams. Especially in the case of German yellowjackets (*Vespula germanica*), they also often nest in the walls of structures. Aerial yellowjacket nests are round gray paper-mâché balls attached to eves and walls or tree branches. The biggest risk with yellowjacket nests is inadvertently disturbing them. A few precautions will reduce this risk. Anyone who is hypersensitive to yellowjacket stings should not mow lawns or preform outside maintenance work, especially during warm weather, without first carefully surveying the area for nests or flight in and out of holes in the ground or walls. A major problem with lawn mowers is their noise that masks any early warning buzzing by defending yellowjackets. Additionally, the heat, motion, vibration, and smell of the mower simulate the cues of a large attacker. The combination is particularly hazardous, as the operator will not notice the yellowjackets until several to many come out and a sting occurs.

Yellowjacket foragers away from their nest also present a risk. The primary reason is that many species of yellowjackets like our food and find our picnics, outdoor snacks, beverages, and garbage cans irresistible. Unlike honey bees that seek only sweets, yellowjackets are fond of both sweets and protein. They readily land on our meat sandwiches, sweet fruits, and will even crawl inside open soft drink cans. In these situations they can be inadvertently consumed or imbibed, whereupon they sting [13]. Especially in the late autumn when colony populations are at their max and natural food resources are becoming scarce, yellowjacket foragers can be a numerous and persistent nuisance. They are not readily shooed away, and swatting or flapping at them only increases the risk of being stung or an individual becoming entangled in hair. In extreme "yellowjacket years," they can become so omnipresent outdoors that enjoying a picnic becomes nearly impossible [3] (Chap. 7).

Paper Wasps

Paper wasps are an enormous, diverse genus of more than 200 species of relatively large social wasps. They are distributed nearly worldwide in areas not extremely cold and construct small open hexagonal-celled nests attached to vegetation and structures or nestled inside rock or other cavities [14]. Paper wasps come in a dazzling variety of colors from pure black, red, or yellow to striped or spotted combinations of these colors plus browns and tans. They are mainly recognized by their shape and habit of flying with their hind legs dangling below their bodies. Their diets heavily, or exclusively, consist of caterpillars, thereby making paper wasps beneficial to gardeners and agriculture. Paper wasps forage for nectar at flowers and for water from various sources including swimming pools, bird baths, and leaky water faucets, but they are not attracted to people or their food.

Paper wasps are most active in spring, summer, and fall, starting with one or several "foundress" females that build a small paper nest in which they raise the first generation of workers. At this stage the wasps rarely mount a defense or sting;

rather, they attempt to fly away and escape. Once the nest population increases, the workers become more defensive, and stinging attacks become more likely. Even with high populations, paper wasps tend not to sting, but instead often mount a series of threatening behaviors [15]. The most common human stinging encounters with paper wasps occur when people directly disturb a mature nest, for example, in abruptly opening a shed door and jarring a nest hanging directly above the door. Entrance door areas are common nesting areas for the wasps and can be problem areas.

Paper wasps never willingly sting when away from their nest. The main concern away from a nest is near water, especially swimming pools. Paper wasps collect water at the edges of pools and sometimes while floating on the open surface. If someone jumps in the pool, wasps can be knocked into the water and sting while struggling to escape. Another problem is putting hands on a wasp when getting out of the pool or in hoisting one's posterior up onto the pool edge.

A few precautions around houses and yards will make stings by paper wasps rare. Simple searches of areas before opening doors or windows or doing maintenance or yard work will help prevent startling the wasps. Likewise, look before leaping into swimming pools.

Hornets

True hornets (*Vespa*) are found predominantly in Asia including Japan and Korea, with one species native to Europe and one species each introduced to Europe and North America, the latter mainly present in the Appalachian and Midwest regions of the USA [2]. Hornets can be viewed as huge oversized yellowjackets in many regards, including much of their natural history. Because they are large, hornets can inject enormous quantities of venom and can cause direct toxic damage in addition to inducing hypersensitivity [16, 17]. Like yellowjackets, hornets build nests underground, often at the base of trees or shrubs, in tree hollows, or as large spherical paper balls attached to branches or walls. Precautions to avoid hornet stings are much the same as for yellowjackets.

Fire Ants

Unlike bees and wasps, fire ants (*Solenopsis* spp.) cannot fly to sting people. But what they lack in flight, they make up in sheer numbers and aggressive defensive behavior. Upon contact with any person or animal, fire ants almost seem to have an attitude of "sting first, ask questions later." This blend of behaviors makes fire ants a menace to inattentive individuals in areas having them.

Fire ants are a group of several dozen species native to the warmer areas of North and South America [18]. The various species are similar in general appearance and

behaviors and can be summarized as: "if you've met one fire ant, you've met them all." Fire ants build large colonies of thousands to hundreds of thousands of ants and eat almost anything that comes their way—live prey, dead animals, seeds, nectar, honeydew, and plant material—and they aggressively defend their territories by attacking intruders. All fire ants sting and their stings hurt. In the USA the two most important fire ant species are the "imported" fire ants, *S. invicta* and *S. richteri*, both transported from South America to Alabama in the first half of the twentieth century. These two species inhabit the Southern USA and Texas with a population also in Southern California. Native species of fire ants are also present in Western USA and constitute minor causes of allergy in areas such as Arizona and New Mexico. Commerce has distributed invasive fire ants including *S. geminata* around much of the world including China, Taiwan, Australia, and many oceanic islands, all places where they have become a problem [19, 20].

Fire ants prefer to live in soft, sandy soil in warm, sunny areas with grass intermixed with other plants, having few other ants competing for space and resources. These areas are disturbed habitats or what ecologists call successional habitats. In nature, disturbed areas are relatively scarce, occurring mainly after fires, floods, powerful storms, major pest/pathogen outbreaks, or huge tree falls that dramatically alter an area. Humans raze land to plant crops, graze cattle and other livestock, culture grass and lawns around dwellings and spaces, and, intentionally or unintentionally, burn areas. Human activity maintains these areas in a de facto state of permanent disturbance, making them perfect for fire ants. In lawns and pastures enormous numbers of colonies can be present, each with a mature colony population around 200,000–300,000. Fire ant colonies live 5–7 years and are active year round.

Precautions to avoid fire ant stings are more complicated than for avoiding bee or wasp stings because fire ants can be so ubiquitous and widespread in the human environment. This partly explains why half the human resident population in fire ant-infested areas is stung yearly, and only 10% or fewer residents in bee or wasp areas are stung yearly [21]. Steps to reduce chances of fire ant stings center on vigilance and some control. Sensitive people should wear shoes instead of flip flops or sandals and stay on paved sidewalks and roads as much as possible. Care is needed in walking on lawns or in open areas with any fire ant mounds avoided. Sitting or resting on the ground can be hazardous—again look before leaping (or sitting). Finally, fire ant mounds in yards where children and adults play or are active might be treated [22].

Other Ants

A variety of ants other than fire ants occasionally sting people causing medical problems. In North America, these include the harvester ants (*Pogonomyrmex* spp.) of the US West and South, the little fire ant (*Wasmannia auropunctata*), and two introduced species: the European fire ant (*Myrmica rubra*), and the Asian needle ant (*Brachyponera chinensis*) [23–25]. Harvester ants, known to induce

hypersensitivity in some patients and are in the subfamily Myrmicinae, are the same subfamily as the fire ants [26]. Also in that subfamily are the European fire ants, an invasive species now also found in Northeastern North America, Vancouver, Canada, and Japan, and little fire ants found in Florida and much of the rest of the tropical world [27]. The Asian needle ant, a native of Japan, Korea, and surrounding areas, is now present in the forested areas of the American Southern Appalachian Region and in New Zealand. Allergic reactions to needle ant stings are commonly reported in its native range and the major venom allergens reported [28]. It is unclear the extent of cross-reactivity, if any, exists between the stings of the three myrmicine ants, though based on sting reactions, the author suspects the venoms of *Pogonomyrmex* and *Myrmica* are cross-reactive.

In parts of Africa and much of the Middle East, the samsum ant (*Brachyponera sennaarensis*) causes some allergic reactions to stings [29]. This small 4–5 mm long black ant can be strongly defensive and packs a surprisingly painful sting for its small size (personal observations).

One would be remiss not to mention the jack jumper and bull ants of Australia. Especially the jumper ants (*Myrmecia pilosula* species group), 12–14 mm-long ants found most abundantly in the southern parts of Australia, which not only deliver painful stings, but constitute a significant cause of hypersensitive reactions [3, 30]. The bull ants, often called bulldog ants, are much larger *Myrmecia* ants that also sting painfully and cause some allergic reactions. The large genus *Myrmecia* is restricted to Australia with a few species in New Guinea and New Caledonia.

Although not reported to induce allergic reactions, the bullet ant, *Paraponera clavata*, might be of interest to clinicians simply because patients may ask about it. Bullet ants are huge, measuring 20–25 mm in length, and deliver the most painful sting of any insect. They are used in puberty rites for some peoples in Amazonas as a test of strength, will, and readiness for manhood (https://www.youtube.com/watch?v=XwvIFO9srUw) [31]. Bullet ants readily sting causing immediate pain ("feels like getting hit with a bullet") that throbs in a seemingly unending series of excruciating waves for 12–24 h. People living in the ant's range of Atlantic Nicaragua to Southern Brazil are well familiar with bullet ants, and their wariness against repeat stings might explain a lack of allergy reports.

Other Bees

Bumble bees are the main concern for bee stings from bees other than honey bees. The 250+ bumble bee species are most abundant in the temperate regions of the northern hemisphere, though a few species live in the New World as far as the southern tropics and temperate zones [32]. Most species construct small underground nests of a few dozen to a hundred or so individuals and are generally docile unless their nest is severely disturbed. Bumble bees do not lose their stings like honey bees, but otherwise their stings are similarly painful, and their venoms are somewhat cross-reactive with honey bee venom [33]. The main precautions individuals should take to avoid bumble

bee stings are to leave nests undisturbed, run to safety if a nest is disturbed, and not to frolic barefoot in lawns or parks where bees might be visiting clover flowers.

Of the 20,000 species of bees, only two other types might be of interest to patients and clinicians. These are the large black carpenter bees (*Xylocopa* spp.) and the small, often grayish sweat bees (Halictidae, mainly *Lasioglossum*). Carpenter bees are solitary species of nearly worldwide distribution that will only sting is grasped or trapped in clothing. Some sweat bees are attracted to human perspiration and will sting if inadvertently pinched. Their stings produce minor pain, but evidence strongly supports that they can induce hypersensitivity [34].

Other Social Wasps

An abundance of social wasps inhabit in the Neotropics and the Old World tropics. These wasps are different from the *Vespula*, *Dolichovespula*, *Vespa*, and *Polistes* addressed previously; nevertheless, most can be defensive and sting. Important among these are the *Polybia*, *Brachygastra*, *Synoeca*, *Parachartergus*, *Apoica*, *Agelaia*, and *Mischocyttarus* of North and South America and *Ropalidia*, *Belonogaster*, and *Parapolybia* of the Old World [4, 35]. Little is reported on the allergenicity of venoms from these species, though cross-reactivity of these venoms with those of *Polistes*, *Vespula*, etc. would not be surprising. In any case, prudence in areas with these wasps is advised.

Solitary Wasps

Most solitary (i.e., nonsocial) wasps cannot, or will not, sting humans. Exceptions are the velvet ants—fuzzy, often brightly colored female wasps in the family Mutillidae—and the spider wasps, including the tarantula hawk (*Pepsis*) in the family Pompilidae. Stings from these wasps are excruciatingly painful, though allergic reactions appear not to have been reported. Likewise, the enormous cicada killer (*Sphecius* spp.), a solitary wasp that appears like a yellowjacket on growth hormone and steroids, not only is not reported to induce allergic reactions, but it is nearly impossible to induce it to sting a person. All of these wasps are best enjoyed for their biology and beauty.

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Chapter 16
Insect Sting Allergy and Mast Cell Disease

Patrizia Bonadonna, Roberta Zanotti, and Franziska Ruëff

Abbreviations

BM	Bone marrow
CM	Cutaneous mastocytosis
HVA	Hymenoptera venom allergy
ISM	Indolent systemic mastocytosis
MC	Mast cells
MIS	Mastocytosis in the skin
MMAS	Monoclonal mast cell activation syndrome
SM	Systemic mastocytosis
VIT	Venom immunotherapy

P. Bonadonna
Allergy Unit, Azienda Ospedaliera Universitaria Integrata di Verona,
Piazzale Stefani 1, Verona 36126, Italy
e-mail: patrizia.bonadonna@ospedaleuniverona.it

R. Zanotti
Section of Hematology, Department of Medicine,
Azienda Ospedaliera Universitaria Integrata di Verona,
Piazzale Scuro 1, Verona 36100, Italy
e-mail: roberta.zanotti@univr.it

F. Ruëff (✉)
AllergieZENTRUM, Klinik und Poliklinik für Dermatologie und Allergologie,
Klinikum der Universität München, Frauenlobstr. 9-11, Munich 80337, Germany
e-mail: Franziska.Rueff@med.uni-muenchen.de

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Definition of Primary Mast Cell Diseases

Mast cell (MC) diseases may be broadly divided into those defined by expansion of clonal mast cells, i.e., primary MC disorders, and those defined by an MC activation without demonstration of clonal MCs; often, however, there is a substantial overlap between those two entities. A mutation of the activating receptor KIT is primarily responsible for the development of mastocytosis. A stem cell factor (KIT ligand) stimulates the MC via a tyrosine kinase receptor (KIT) to growth and differentiation. Up to now, more than ten different mutations have been found. The mutation results in an activation of the receptor without the presence of a ligand thereby stimulating proliferation and enhancing survival of MCs. Another feature of mast cell diseases is the expression of certain adhesion molecules (CD2, CD25) on the MC surface; such adhesion molecules cannot be found on normal mast cells [1, 2]. The majority of MC disorders associated with Hymenoptera venom allergy (HVA) are primary MC disorders that include mastocytosis and monoclonal MC activation syndrome (MMAS) [3, 4].

Mastocytosis

In more than 90% of the patients, mastocytosis is associated with a point mutation of the proto-oncogene c-KIT. The most common mutation is located in codon 816 of exon 17 of the KIT gene (D816V); this and the majority of other, much less frequent KIT mutations involving codons 815, 816, 817, 820, and 839 [5, 6], directly affect the enzymatic site of the receptor at the TK2 activation loop, inducing conformational changes which are associated with subsequent constitutional activation of KIT in the absence of a dimerization of the receptor [7]. Activation of KIT allows the proliferation of MC precursors and enhances survival of mature MC.

The diagnosis and classification of mastocytosis include the identification of neoplastic MCs on the basis of their morphological, immunophenotypic, and molecular characteristics and of the well-established World Health Organization [WHO] criteria [8]. SM will be diagnosed if the patient presents with the major criterion plus one minor criterion, or at least with three out of four minor criteria (Table 16.1) [8]. The WHO classification revised in 2016 defines seven variants of mastocytosis (Tables 16.2 and 16.3) [9]; among those, cutaneous mastocytosis (CM) predominates in children and indolent systemic mastocytosis (ISM) in adults.

Besides the increase of MC content in various tissues, there are several other abnormal features of MC, which patients may demonstrate who suffer from a systemic mastocytosis (SM), and which are absent in MCs from healthy subjects. Overexpression of several adhesion- and activation-related antigens such as CD2, CD25, CD63, CD69, and CD203c was shown on MCs of patients with SM. Surface membrane receptors involved in the binding of complement components and complement-mediated cell activation are also overexpressed in patients with SM [10]. These changes may cause a dysfunction of the MC.

Table 16.1 WHO diagnostic criteria for systemic mastocytosis (SM)

The diagnosis of SM is established when at least one major and one minor or at least three minor criteria are present	
Major	Multifocal dense infiltrates of MC in bone marrow (BM) sections or other extracutaneous organ(s) (>15 mast cells (MCs) in aggregate)
Minor	(a) MCs in bone marrow or other extracutaneous organ(s) show an abnormal (spindle-shaped) morphology (>25%) (b) *KIT* mutation at codon 816 in extracutaneous organ(s). In the majority of cases, the mutation is D816V (c) MCs in bone marrow express CD2 and/or CD25 (d) Serum BTC >20 µg/l (cannot be applied to patients who have associated clonal hematological non-mast cell lineage disease, i.e., AHNMD)

Table 16.2 WHO classification of mastocytosis (revised in 2016) [9]

	Abbreviation	Definition
1. Cutaneous mastocytosis	CM	Exclusive involvement of the skin
2. Systemic mastocytosis	SM	
(a) Indolent SM • Isolated bone marrow mastocytosis*	ISM	Meets criteria for SM. No "C" findings and <2 "B" findings (Table 16.3). No evidence of AHNMD *ISM sub-variant with bone marrow involvement, but without skin involvement
(b) Smoldering SM	SSM	As ISM, but with two or more "B" findings, and no "C" findings
(c) Systemic mastocytosis with an associated hematological neoplasm	SM-AHN	All variants of SM associated to another hematological neoplasm. AHNMD and AHN can be used synonymously
(d) Aggressive SM	ASM	Meets criteria for SM. One or more "C" findings. No evidence of mast cell leukemia
(e) Mast cell leukemia	MCL	Meets criteria for SM. Bone marrow biopsy shows a diffuse infiltration, usually compact, by atypical, immature mast cells. BM aspirate smears show ≥20% mast cells. In typical MCL, mast cells account for ≥10% of peripheral blood white cells
3. Mast cell sarcoma	MCS	

Monoclonal Mast Cell Activation Syndrome

Some patients display symptoms of MC mediator release but do not strictly fulfill the WHO criteria for SM despite having KIT-mutated clonal MCs, and expressing CD25 on bone marrow (BM) MCs: these patients have been classified by various terms including monoclonal MC activation syndrome [MMAS] [11–15], primary MC activation syndrome [MCAS] [16], or clonal MC activation disorders [c-MCAD] [3, 17].

Table 16.3 Definition of B and C findings

"B" findings	A. BM biopsy showing >30% infiltration by MC (focal, dense aggregates) and/or serum total tryptase level > 200 ng/mL B. Signs of dysplasia or myeloproliferation, in non-MC lineage(s), but insufficient criteria for definitive diagnosis of an AHNMD, with normal or slightly abnormal blood counts C. Hepatomegaly without impairment of liver function, palpable splenomegaly without hypersplenism, and/or lymphadenopathy on palpation or imaging
"C" findings	1. Bone marrow dysfunction manifested by one or more cytopenia(s) (ANC < 1.0 × 109/L, Hgb < 10 g/dL, or platelets < 100 × 109/L), but no obvious non-mast cell hematopoietic malignancy 2. Palpable hepatomegaly with impairment of liver function, ascites, and/or portal hypertension 3. Skeletal involvement with large osteolytic lesions and/or pathological fractures 4. Palpable splenomegaly with hypersplenism 5. Malabsorption with weight loss due to gastrointestinal mast cell infiltrates

Abbreviations: *MC* mast cell(s), *ANC* absolute neutrophil count, *Hgb* hemoglobin, *BTC* baseline serum tryptase concentration

Diagnosis of Primary Mast Cell Diseases

Baseline Serum Tryptase Concentration (BTC)

Tryptase is the main serine protease contained in the secretory granules of human MC, and it is produced almost exclusively by MC, with the exception of a small amount produced by basophils; therefore, BTC is an easily measurable in vitro marker of the MC diseases [18]. The two isoenzymes that are most abundantly expressed in and secreted by mast cells are α- and β-tryptase. Mature β-tryptase is stored in mast cell granules as an enzymatically active protease and is released only after mast cell stimulation. The tryptase which can be found in serum without a specific mast cell activation (baseline tryptase) consists of enzymatically inactive pro-tryptases.

It is assumed that BTC reflects the mast cell burden of the body. There are exceptions such as patients with mastocytosis who develop diffuse bone sclerosis being associated with a great increase in BTC unrelated to the true MC load [19, 20].

A BTC ≥ 20 μg/L represents one minor WHO criteria to diagnose SM. However, a normal BTC does not rule out a diagnosis of SM [21–23]. Nineteen of 57 patients with ISM (33.3%) had a BTC < 20 μg/l, and 10 of the 19 patients (17.5% of the total cohort) presented with a BTC within the 95th percentile [22]. On the other hand, increased BTC is not an exclusive symptom of SM but may also be associated with many hematologic malignancies, particularly of myeloid origin, and with nonneoplastic condition such as end-stage chronic renal failure, onchocerciasis, and chronic urticaria. Therefore, SM can neither be excluded by a normal BTC nor can it be absolutely proven by an increased BTC.



Fig. 16.1 Erythematous spots of 1–2 mm diameter (type telangiectasia macularis eruptive perstans, TMEP) in a 53-year-old male with insect venom allergy. Skin biopsy showed slightly increased number of mast cells. Baseline serum tryptase 40.4 µg/l. Bone marrow biopsy refused by the patient

Skin Examination

A thorough examination of the skin is required to detect the cutaneous lesions. Upper legs, buttocks, and lateral trunk are frequently involved, whereas palms, soles, and face are mostly spared. There are a great variety of MS-associated skin lesions ranging from hardly visible erythematous spots (Figs. 16.1 and 16.2) to typical maculopapular (urticaria pigmentosa-like) skin lesions (Fig. 16.3), and to a dense infiltration of the skin (Fig. 16.4). Non-dermatologist may mistake pigmented types of MIS for freckles which, however, should show a UV-dependent distribution. Physical stimuli (e.g., mechanical, thermic) may induce a mast cell release which results in itching and swelling of the skin lesions upon rubbing. A positive, so-called Darier's sign (see Fig. 16.5) may be easily obtained by a simple physical examination and is highly suggestive of MIS. Histological examination of a skin biopsy confirms the diagnosis. MIS in adults is highly predictive for SM [22], which, however, cannot be excluded by the absence of skin lesions.



Fig. 16.2 Minor skin lesion, urticaria pigmentosa, in 49-year-old female with ISM and *Vespula* venom allergy. Baseline serum tryptase 177 µg/l



Fig. 16.3 Easy visual diagnosis: urticaria pigmentosa in a 53-year-old male with typical brownish maculopapular skin lesions

Screening for a D816V KIT Mutation in the Peripheral Blood

Using highly sensitive and quantitative PCR-based assays, KIT D816V can be detected in peripheral blood leukocytes from many patients with SM; positive results may be obtained in 43–100% of the patients [24–27]. The demonstration of a KIT D816V mutation in blood cells is suggestive of SM. As suggested by the European Competence Network on Mastocytosis, the allele-specific oligonucleotide-qPCR method should be the most sensitive method to detect an ISM [28, 29]. This method, however, may only identify 66% of patients truly suffering from ISM, who

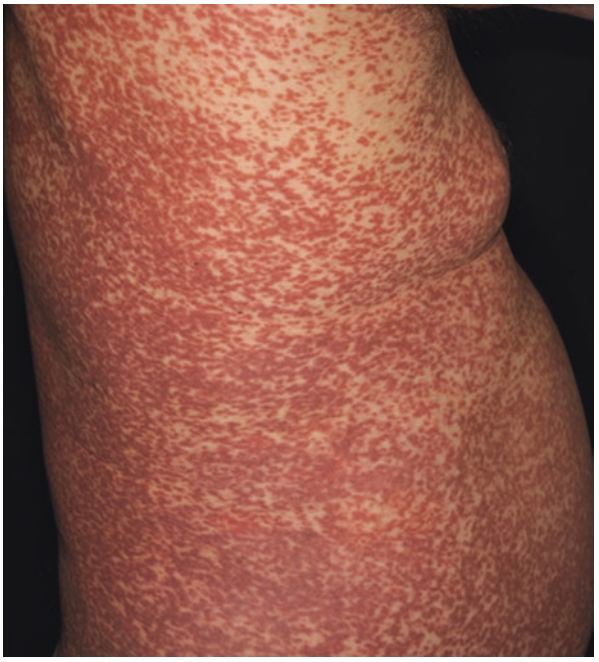


Fig. 16.4 Dense in part diffuse cutaneous infiltration of the skin in a 54-year-old male with ISM. Baseline serum tryptase 64.8

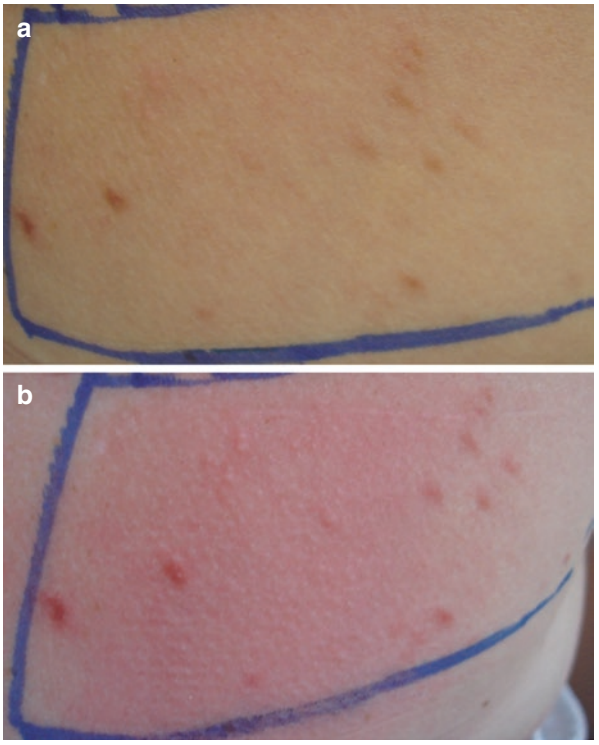


Fig. 16.5 Positive Darier's sign. Urticaria pigmentosa before (**a**) and after (**b**) mechanic stimulation

do not have an MIS [27]. Moreover, a significant percentage (3–5%) of these patients carry other KIT mutations being distinct from D816V; consequently, in these patients, SM cannot be diagnosed with this method.

Bone Marrow (BM) Evaluation

A BM biopsy should be considered in patients with or without MIS and/or strong suspicion for SM [21, 28].

Bone Marrow Histology

In BM biopsies (or in other extracutaneous organs), the histological diagnosis of SM requires the identification of compact and multifocal infiltrates of MCs (at least 15) (major criterion). The identification of atypical MCs within these aggregates is sufficient to diagnose SM (Fig. 16.6a–c). The diagnosis may be confirmed by immunohistochemical techniques, in particular by demonstrating an expression of MC tryptase, c-kit, or CD117 and CD25 [8]. Very often, however, the major histological criterion is not met, as only small clusters or isolated scattered MCs are detectable in the BM (Fig. 16.6d–f). In such patients, other sensitive tests are needed to identify the criteria essential for a diagnosis of SM, such as the flow cytometry (FC) and the search for mutation in codon 816 of the *KIT* gene [30, 31].

Bone Marrow Smear Morphology

In the BM smears from SM patients, which have been stained with May-Grünwald-Giemsa or Toluidine blue, MCs show a variable cellular atypia: a spindle-shaped cytoplasm and/or protrusions, an eccentric oval nucleus, and a hypogranulated cytoplasm with a focal accumulation of granules or without fusion of the granules (Fig. 16.7). In some patients, it is possible to find MCs with monocytoid bi- or multilobed nuclei being typical for aggressive diseases, or round mature MCs [32].

MC Immunophenotyping on Bone Marrow Aspirate

MCs can be easily identified through a multiparametric FC study using a minimum panel of markers (Fig. 16.7). Normal MCs are characterized by a strong expression of CD117 and of CD45 and are negative for CD34 [33]. Pathological MCs are distinguishable by an aberrant expression of CD25 and of CD2 (Fig. 16.8). The number of pathological MCs (CD45-positive cells) present in the BM of ISM patients is generally <2%, ranging from 0.002 to 1.7% [30, 33]. Especially in patients with an ISM without skin lesions, clonal MCs represent a very small proportion of all

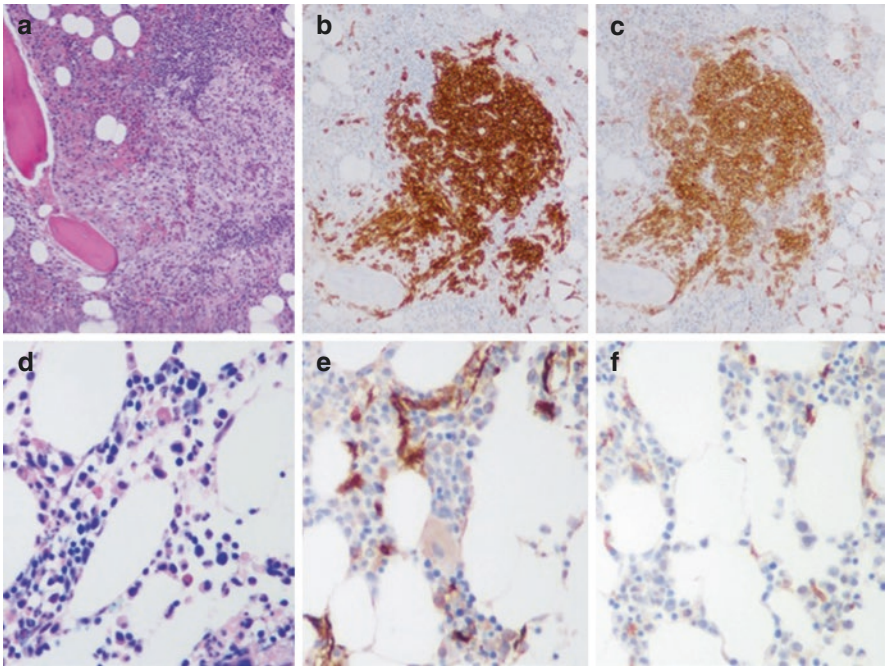


Fig. 16.6 (**a**–**f**) Histopathology of systemic mastocytosis on bone marrow biopsy; histopathology of systemic mastocytosis on bone marrow biopsy; (**a**) low power view showing huge mast cells aggregate in a paratrabecular area (H&E, 10×); (**b**) low power view showing a mast cell aggregate, composed of round and spindly mast cells: mast cells are positive for CD117 by immunohistochemistry (anti-CD117 antibody revealed by peroxydase-DAB method, 10×); (**c**) low power view showing mast cells paratrabecular aggregate positive for CD25 (anti-CD25 antibody revealed by peroxydase-DAB method, 10×); (**d**) high-power view showing few spindle-shaped mast cells (Giemsa, 40×); (**e**) CD117-positive spindle mast cell in interstitial location (anti-CD117 antibody revealed by peroxydase-DAB method, 40×); (**f**) CD25-positive spindle mast cell in interstitial location (anti-CD25 antibody revealed by peroxydase-DAB method, 40×) [Courtesy of Dr. Alice Parisi]

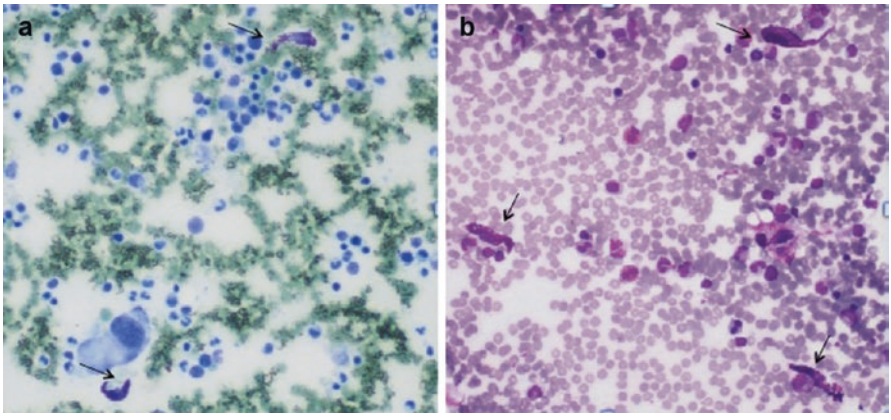


Fig. 16.7 MC cytology. Bone marrow smears stained with toluidine blue (**a**) and May-Grünwald-Giemsa (**b**) show low percentage of mature atypical MC type I [indicated by *arrows*) (Courtesy of Dr. Alice Parisi]

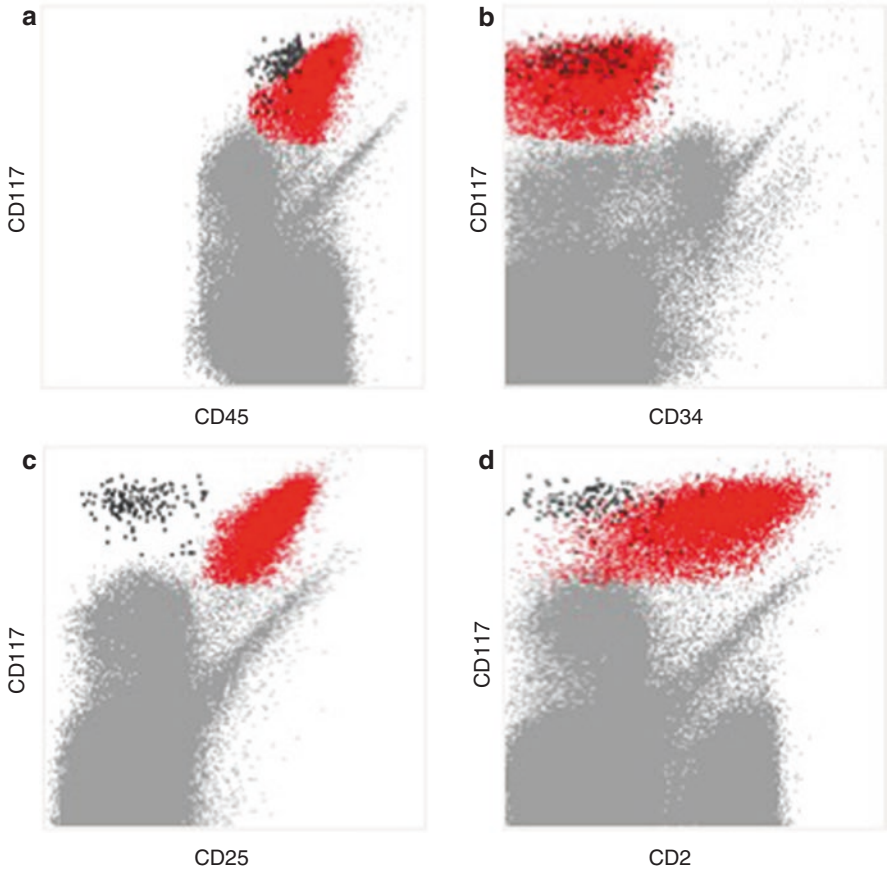


Fig. 16.8 Flow cytometric analysis of BM MCs. MCs were identified as CD117++/CD45+/CD34+ cells (**a** and **b**, *dot plots*). CD25 and CD2 antigens were clearly aberrantly expressed on neoplastic MCs (*red*) but not on normal MCs (*black*; **c** and **d**, *dot plots*) [Courtesy of Dr. Omar Perbellini]

nucleated BM cells [3, 13]; therefore, it is mandatory to use specific immunophenotypic gating strategies for detecting MCs of which corresponding frequencies in a sample may be as low as one out of $\geq 10^6$ cells [30].

Evaluation of a KIT Mutation in the Bone Marrow

Since in BM the neoplastic MC burden is frequently very low, especially in ISM patients without skin involvement, it may be sometimes difficult to analyze the mutational status of *KIT*. Direct sequencing by a Sanger method is not sensitive enough. Guidelines suggest the use of a reverse transcription polymerase chain reaction [RT-PCR] with restriction fragment length polymorphism, peptide nucleic acid PCR, and allele-specific quantitative PCR [21, 29].

Epidemiology and Clinical Presentation of Mastocytosis

Mastocytosis can affect any age group; the gender distribution is approximately equal [33]. In the majority of patients, mastocytosis appears sporadic, although familial clusters have been reported [34, 35]. Childhood mastocytosis is often limited to the skin (cutaneous mastocytosis, CM), while in adults the predominant type involves one or more extracutaneous organs, i.e., SM affects the BM, gastrointestinal tract, lymph nodes, or spleen, and may or may not be associated with MIS. In contrast to adult patients, the majority of the children will outgrow mastocytosis [36]. Recent studies estimate that the prevalence of SM in European adults varies from 10 to 13 patients per 100,000 inhabitants [37, 38].

Most commonly, a diagnosis of MIS or ISM has not been established at the time when a patient presents for the first time with symptoms due to an insect venom allergy [13, 39]. This phenomenon indicates that the majority of patients simultaneously suffering from an ISM and a HVA are otherwise healthy and have not taken notice of their mast cell disease before.

Mastocytosis may show a wide variety of clinical manifestations. Skin lesions may disturb the patient for cosmetic reasons. Dysfunction of MCs may cause an inappropriate release of mediators and may induce cutaneous symptoms, e.g., pruritus, urticaria, angioedema, and flushing. Gastrointestinal (e.g., nausea, vomiting, abdominal pain, diarrhea) or cardiovascular symptoms (anaphylaxis) may occur. Hymenoptera stings represent the most common trigger of anaphylaxis in subjects suffering from a clonal mast cell disorder; besides that, particular drugs, alcohol, "stress," cold, heat, or infection may act as mast cell releasing factors [15, 40, 41]. Osteopenia and (manifest) osteoporosis, and in some patients osteosclerosis, are important secondary diseases associated with SM [42]. An early diagnostic work-up which includes bone density measurements and X-ray of total spine and pelvis will be required to identify affected patients.

Hymenoptera Venom Allergy and Clonal Mast Cell Disorders

Prevalence

The association between HVA and mastocytosis was initially described in case reports and case series of adult patients presenting with an urticaria pigmentosa [43–46]. In HVA patients, the first systematic evaluation for monoclonal mast cell diseases was done by Bonadonna et al. revealing an ISM in 21 (5.5%) and a monoclonal mast cell activation syndrome in 9 (2.4%) of 379 consecutive patients [13]. In various cohorts, the frequency of clonal mast cell diseases among adults with HVA ranged from 1 to 7.9% [47]. In a retrospective evaluation of sting challenge tests in 1532 patients on VIT, 98 (6.4%) patients had been diagnosed with a mast cell disease using clinical indicators such as an adult-onset MIS and/or a BTC > 20.0 μg/L [39]. The true incidence of monoclonal mast cell diseases in patients with HVA will probably remain unknown as the complete diagnostic

arsenal is only applied if there is a suspected mast cell disease, and bone marrow biopsies in patients without any sign of a mast cell disease are not justifiable.

Several authors found that the majority of patients with HVA who are diagnosed with mastocytosis actually present with an ISM lacking skin lesions [3, 13, 17, 21, 48, 49]. Others, however, found that MIS may occur in more than 80% of HVA patients showing signs of a mast cell disease [39]. These discrepancies may either be explained by different patient populations, by differences in the selection of patients for bone marrow evaluation, or by variable standards used for skin examinations.

The type of mast cell disease, which is typically associated with HVA, is an ISM or a monoclonal mast cell activation syndrome [13, 22]. These patients have a normal life expectancy and are usually healthy except for mediator release due to other triggers or disorders of the bone density.

Compared to the average population, the prevalence of HVA is increased among patients with mastocytosis: in a questionnaire-based evaluation of 420 patients with SM allergic sting, reactions were reported by approximately 25% of the patients [50]. Case series of patients with mastocytosis found a prevalence of HVA in 5% [51], 20% [52], and 27% of the patients [40]. Surprisingly, HVA seems to be absent in patients who suffer from aggressive subtypes of SM harboring the highest mast cell load [53, 54]. In addition, in patients with SM initially presenting with HVA, a progression to an aggressive mastocytosis has not yet been reported [55]. However, although very rare, development of an associated hematological malignancy may occur [4]. In contrast to adults, children having a mastocytosis disorder do not seem to be at increased risk for HVA [40, 56].

Pathophysiology

MCs are part of the innate immune system and are important for detoxifying venoms, e.g., from snakes [57]. Carboxypeptidase A which is one of the proteases contained in MC appears to be the key enzyme which is responsible for the elimination of sarafotoxin. The latter is the most potent toxic component of *Atractaspis engaddensis* (Israeli mole viper). A controlled MC activation and degranulation are of central importance for mediating the antivenom effect, and these MC functions must be regarded by nature as beneficial in mammals which may be protected but not harmed by MCs. MC activation may be mediated by Fcε receptor-dependent and Fcε receptor-independent mechanisms. Hymenoptera venoms are potent releasers of mast cell compounds via IgE-mediated and non-IgE-mediated mechanisms [58–62]. Bridging of Fcε-receptors-bound allergen-specific IgE antibodies allows a fast release of various mediators from MCs. In comparison to Fcε receptor-independent mechanisms, Fcε receptor-dependent pathways are significantly more important when MCs are activated by Hymenoptera venom.

Various venom proteins may induce the production of IgE antibodies. A protective function of MC, which should physiologically lead to a fast release of detoxifying compounds, is also involved in Hymenoptera venom anaphylaxis. It is likely that an increased number of effector cells participating in anaphylactic reactions contribute to the particular risk of HVA. Furthermore, it is highly likely that in patients with SM, a variety of immunophenotypic particularities may change the function of MCs, thereby simultaneously increasing the risk for severe anaphylactic events in these patients [63].

Indication for a Screening for Mast Cell Diseases in Patients with Hymenoptera Venom Allergy

The recommendation to screen patients with HVA for features of mast cell diseases is only valid for adult patients. Statistically, it has been found in children that severe sting reactions are associated with higher BTCs; however, since BTCs still were within the normal range, these findings do not indicate mast cell disease [64]. Therefore, at present, there is no evidence to screen children for mast cell diseases if they just present with an HVA.

- We recommend measuring BTC in all adult patients with a history of systemic sting reactions (at least in those patients presenting with symptoms not only involving the skin). However, even a BTC within the 95th percentile cannot exclude a mast cell disease in patients with HVA and hypotension due to the sting reaction: in 16 of 22 patients who did not have MIS, and whose BTC was <11.4 mg/L, ISM could still be diagnosed by a BM examination; one of these 22 patients had a monoclonal MC activation syndrome [23]. A significantly increased BTC may also indicate hematologic diseases other than ISM and should, therefore, stimulate a broader diagnostic work-up, particularly in those patients not having an MIS.
- A screening for mastocytosis should be done in those patients who present with an increased BTC or with a history of severe sting reactions (anaphylactic shock, near-fatal reactions) or who have had an uncommon course or outcome of VIT. Screening should start with a skin examination. Almost all adult patients presenting with a MIS actually suffer from SM [22]. However, skin lesions may be frequently absent in SM patients who present with Hymenoptera venom anaphylaxis [13].
- The so-called liquid-based biopsy wants to detect genetic information (KIT mutations) in peripheral blood cells. This test has, however, a limited sensitivity in patients without skin lesions [27].
- In patients demonstrating a markedly increased BTC (>25–30 μg/l), a BM biopsy is usually recommended [28].
- If patients refuse a BM biopsy, a bone density measurement should be performed at least.

Table 16.4 The REMA Score (Red Española de Mastocitosis) [3]: Proposal for a screening method for the presence of clonal mast cells in patients presenting with anaphylaxis in the absence of mastocytosis in the skin

Variable	Score points	
Gender	Male	+1
	Female	−1
Clinical symptoms	Absence of urticaria and angioedema	+1
	Urticaria and/or angioedema	−2
	Presyncope and/or syncope	+3
BTC	<15 μg/l	−1
	>25 μg/l	+2

Abbreviation: *REMA* Red Española de Mastocitosis (Spanish Network on Mastocytosis)
Proposal for a screening method for the presence of clonal mast cells in patients presenting with anaphylaxis in the absence of mastocytosis in the skin

Patients with normal BTC levels not having an MIS represent a diagnostic challenge. Experts are divided into those being more and those being less aggressive concerning the indication for a BM biopsy. Thus, it has been recommended to perform BM biopsies in all patients not showing an urticaria or angioedema during a very severe sting reaction, irrespective from their magnitude of BTC [13, 23]. Others have been more selective suggesting the use of a score (REMA score) to identify possible candidates for a BM biopsy. The REMA score was originally proposed by the Spanish Competence Network on Mastocytosis (Red Española de Mastocitosis) (see Table 16.4) and will yield high score values for those patients who are male, who have had a severe systemic (presyncope or syncope), but not a cutaneous reaction (pruritus, hives, or angioedema) after an insect sting, and who present with a high BTC (>25 μg/l). This score has been shown to identify patients with a clonal MC disorder at a sensitivity and specificity of 91% and 75%, respectively [3]. The authors who defined the REMA score recommend that BM biopsies should be performed in all patients having a REMA score ≥ 2. Some aspects, however, may limit the use of the REMA score. For example, score values may not be correct since skin reaction symptoms such urticaria or angioedema can be overlooked during a severe sting anaphylaxis thereby falsely increasing the score value. Finally, there are some experts suggesting any further work-up (including a BM biopsy) only in those patients presenting with an increased BTC (>20 μg/l), and irrespective of their degree of sting reaction [39].

Currently, significant uncertainty still surrounds many aspects of the diagnostic work-up to be done in patients who suffer from HVA, and in whom SM might be a likely co-disease. Any invasive diagnostic procedure has to be justified by its therapeutic relevance. If a liquid biopsy or a bone marrow evaluation is not possible, BTC may be used as surrogate parameter to identify patients at particular risk. It has been shown that, irrespective of the presence of a mast cell disease, an increased BTC is a predictor of particularly severe sting reaction prior to VIT [65, 66] and of treatment failure (at least in patients allergic to Vespula venom [67]). Furthermore, the combination of an increased BTC (>20 μg/l) with an MIS is associated with VIT failure [39].

Impact of Mastocytosis for HVA

Severity of Sting Reactions

Mastocytosis is a risk factor of its own for severe systemic allergic reactions. Patients with mast cell diseases, who suffer from HVA, develop life-threatening sting reactions after a field sting at a much greater frequency than allergic patients who do not have a mast cell disease [13, 45, 52]. If consecutive patients with HVA are screened for MIS, a significant association between the severity of the sting reaction and the presence or the absence of SM will be found [45]: About 30% of patients who had developed a severe circulatory shock (including loss of consciousness, $n = 37$) will simultaneously demonstrate an MIS (indicating an SM in adults). In patients who only had a mild ($n = 39$) or a moderate ($n = 39$) sting reaction, MIS was significantly less common (about 3% of the patients).

Furthermore, sting reactions may already be fatal at the first sting if the patient simultaneously suffers from an HVA and an SM [68]; therefore, the German Association of Allergy and Clinical Immunology (DGAKI) recommends to all adult patients with mastocytosis that they carry an adrenaline autoinjector even if they had not yet experienced a systemic sting reaction [69].

The majority of SM patients will suffer from a Vespula venom allergy [47]. This particular association is indirectly reflected by several findings: thus, several fatal reactions have been reported in SM patients after a Vespula sting [44, 68, 70], and severe, potentially life-threatening field sting reactions are significantly more common in patients with a Vespula than with a honeybee venom allergy [71, 72].

Safety of VIT

Initially, a review on HVA in patients with mastocytosis reported a high rate (24%) of severe side effects during VIT; one-third of these reactions had to be treated with adrenaline [47]. However, these observations could not be confirmed by subsequent studies examining larger cohorts or case series.

Thus, VIT was found to be safe in nine [73] or ten patients [74] presenting with a MIS or an ISM, respectively. In a large cohort of 84 SM patients, the induction phase of VIT was associated with adverse reactions in ten patients, of whom no one required an epinephrine injection and/or a hospitalization [75]. These data perfectly agree with those of other authors [Ruëff unpublished data].

Adverse reactions, if they occur in SM patients, may be such that VIT has to be stopped in individual patients [76, 77]. Stopping VIT in SM patients, however, is controversial because these patients are at a particularly high risk for future anaphylactic reactions to Hymenoptera stings. To overcome this dilemma, several therapeutic options are available. Thus, SM patients not tolerating VIT can be treated by a combination of omalizumab and an increased maintenance dose. Recently, this combination therapy was shown to induce a durable tolerance of VIT in a series of

five SM patients who had been intolerant to VIT before. Effects persisted even after omalizumab had been discontinued [78].

SM patients requiring a lifelong VIT may sometimes benefit from an extension of the maintenance interval (up to 12 weeks) thereby improving their VIT compliance. On the contrary, it is also possible that an extension of the maintenance interval (for whatever reason) increases the risk for systemic reactions due to VIT. Shortening the intervals between the injections may then reestablish VIT tolerance [Ruëff et al, unpublished data].

Efficacy of VIT

According to a systematic review, efficacy of VIT is reduced in SM patients: only 72% of 82 SM patients were protected if re-stung during VIT [47]. Subsequent studies, however, could not reproduce such low protection rates in SM patients.

Bonadonna et al [75] studied 50 SM patients who had had field re-stings (95 episodes, 1–6 stings) during maintenance VIT 2 months to 7 years after VIT had been started. The authors reported a protection rate of 86%. In a retrospective evaluation of sting challenge tests in 1532 patients on VIT, Ruëff et al. [39] found that a clinically suspected SM (adult-onset MIS and/or a baseline tryptase level of >20 μg/L) was a significant (odds ratio 2.74) albeit not the strongest predictor for VIT failure.

Although standard VIT is effective in SM patients, it may be advantageous to use an elevated maintenance dose to protect individual patients. Importantly, the vast majority of patients in whom standard VIT has failed (including patients with mastocytosis) will be protected if the maintenance dose is increased [75, 79].

In the absence of SM, VIT-induced protection will be permanent in the majority of patients with HVA, and the therapy may be safely stopped after 5 years if several criteria are fulfilled. For SM patients, however, data on VIT duration are sparse. Case reports showed that several patients with mastocytosis died after VIT had been discontinued suggesting lifelong therapy [44, 70].

Practical Recommendations for Managing Patients with a Hymenoptera Venom Allergy Simultaneously Suffering from an SM

A thorough diagnostic work-up should be done soon after sting anaphylaxis. If patients present without atopy, they may soon loose venom-specific IgE antibodies, thereby making a precise identification of the culprit venom more difficult. As patients with a mast cell disease and an HVA are at particular risk for very severe anaphylactic reactions (including a fatal outcome or near-fatal reactions leading to a permanent disability due to, e.g., hypoxic brain damage), VIT is of utmost

importance for those patients. If there is a double sensitization, and even if the patient has a history of only one sting reaction, double VIT should still be considered. Treatment with honeybee venom has been found to be less effective than VIT with Vespula venom [39]. Therefore, SM patients with a honeybee venom allergy should be treated with an elevated maintenance dose (usually 200 μg) right from the beginning of VIT. An increased venom dose may also be considered in SM patients with a Vespula venom allergy who present with additional risk factors (e.g., intense insect exposure, history of a near-fatal reaction). In all patients with mastocytosis, it is strongly recommended to assess the efficacy of VIT by sting challenge tests to identify those who are not protected [39]. In case of a VIT failure, VIT should be continued using an increased dose. In SM, lifelong VIT is recommended. Still there are many open questions regarding the optimal treatment of HVA in patients with mastocytosis. Further studies could better clarify these open issues.

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Chapter 17
Insect Allergy: Unanswered Questions, a Postscript

James M. Tracy

"My entire life consisted of musings, calculations, practical works, and trials. Many questions remain unanswered; many works are incomplete or unpublished. The most important things still lie ahead." Konstantin Tsiolkovsky

Abbreviations

VIT	Venom immunotherapy
BAT	Basophil activation test
ST	Skin test
HVA	Hymenoptera venom allergy

Every story has a beginning and ending. For me the beginning clearly centers around training in San Antonio, Texas, and the imported fire ant (IFA). As a native Minnesotan, I had never paid much attention to this prolific and potentially lethal creature. However, as a first-year fellow looking for novel work and with training as a zoologist with previous experience as a biologist with the Food and Drug Administration, chasing these small creatures seemed interesting. As such it has become the center piece of my professional career. Beginning with an invitation by my colleague Jeffery Demain and encouragement by my then chief and now co-editor Ted Freeman, we set out to determine sting incidence and IgE conversion rate for previously unexposed individuals over a 6-week exposure period [1]. This has led to many interesting opportunities writing and discovering the family

J.M. Tracy, D.O., F.A.C.A.A.I., F.A.A.A.A.I.
Division of Allergy and Immunology, Department of Internal Medicine, Creighton University School of Medicine, Omaha, NE, USA

Allergy, Asthma and Immunology Associates, P.C.,
2808 South 80th Ave, Suite 210, Omaha, NE 68124, USA
e-mail: jtracy@allergynebraska.com

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Hymenoptera. As a side note, as we crafted this book, our working group for Joint Task Force for the Insect Allergy Practice Parameter, many who are contributors for this book, were busy updating our work [2].

The playwright Tennessee Williams tells us, "Life is an unanswered question, but let's still believe in the dignity and importance of the question." Such is the case with insect allergy, and there are many questions. Anaphylactic reactions to stinging insects have been a recognized cause of significant morbidity and potential lethal consequences for many years. However, our understanding of its biology, natural history, and treatment was not readily apparent until the latter half of the twentieth century [3]. One of the goals of this volume was to ask the critical questions and to systematically review the state of our current understanding of the many aspects of insect allergy. To an allergist and immunologist, the stinging Hymenoptera remains the center piece both in terms of diagnosis and management. However, we have taken this opportunity to provide an even broader look at insect biology including mosquito allergy, allergic reactions to biting insects, arthropods, and human disease. However, as far as we have come in our understanding, there remain questions unanswered.

Mast Cell Disease and Hymenoptera Venom Allergy

In recent years' serum tryptase, mastocytosis, occult mast cell disease, as well as mast cell activation syndrome have assumed a significant and emerging role in its relationship with stinging insect allergy management. As Bonadonna et al. point out in Chap. 16 of this work, up to five percent of adults with Hymenoptera venom allergy may also suffer from clonal mast cell disease. We know that the impact of the mast cell is significant, affecting severity of reactions as well as safety and effectiveness of venom immunotherapy. What is the mechanism of this unique relationship between Hymenoptera allergy and mast cell disease? We know that this is a unique relationship compared to other common causes of anaphylaxis such as drugs and foods [4, 5]. So why is this so? Does venom provide certain unique allergen binding opportunities? Some have observed that in cases where the precise venom IgE is not identified, omalizumab stabilized the mast cell in a protective manner [6, 7]. Our understanding of omalizumab in mast cell stabilization may or may not provide additional insights. However, as the authors of Chap. 16 remind us, "Still there are many open questions regarding the optimal treatment of HVA in patients with mastocytosis. Further studies could better clarify these open issues."

As discussed by McKenna and Brown in Chap. 10, honeybee allergy when compared to other Hymenoptera carries additional diagnostic and therapeutic challenges, and this includes the relationship between mast cell disease and honeybee allergy discussed in Chap. 16. Additionally, Bonadonna et al. suggest in Chap. 16 the use of sting challenges as part of the assessment of venom immunotherapy especially in honeybee allergy. Though a very useful measure in determining effectiveness and perhaps the gold standard of investigational work, as Hamilton in Chap. 5 points

out, there are many number of logistical challenges. Additionally, there are considerable regional difference in its practical application. Though the use of sting challenge to assess venom immunotherapy effectiveness is common in some regions of Europe, in others it is not. In North America, its use is considered primarily investigational. Regardless, it is a useful tool to measure overall effectiveness of venom immunotherapy. So what is it that makes honeybee allergy so unique, especially in its relationship with mast cell disease?

Diagnostic Challenges: History Positive, but Venom Testing Negative

Venom immunotherapy can provide up to 95–98% protection against future systemic reactions from stinging Hymenoptera. To initiate this course of treatment requires a reliable history of a systemic allergic reaction and the determination of venom-specific IgE. Currently and commonly available diagnostic tools, including skin testing and newer in vitro methods, appear to be complementary in the detection of venom-specific IgE in 95–98% of individuals who suffer a systemic reaction to a Hymenoptera sting [9]. It is this 2–5% of individuals that provide a specific management challenge. There are many questions. What accounts for the 2–5% of individuals with a compelling history of a systemic reaction? Is it a problem of diagnostic testing sensitivity using currently available methods? Is it somehow related to a comorbid condition such as occult mast cell disease or other non-IgE-mediated event? Or is it that our commercially available venoms used for testing may not include the culpable and relevant Hymenoptera species and necessary venom components?

Once an appropriate history of systemic sting reaction is determined, skin testing remains the primary initial means to demonstrate specific IgE. Though diagnostically sensitive, highly specific, and cost-effective compared to in vitro methods, they are not perfect. Newer testing methods show promise but are not widely available. Dialyzed venoms, though again, not widely available show exceptional capability at increasing sensitivity with testing. Recombinant methods demonstrate benefits with regard to diagnostic specificity [10, 11]. As mentioned in Chap. 5, basophil activation testing (BAT) may also be useful in that it remains the only test that assesses functional activity rather than a demonstration of venom-specific IgE. However, it is considered to be less sensitive than that of intradermal skin testing [12]. Though not widely available, will any of these tests meet the regulatory standards for wide spread commercial approval?

As we consider the critical diagnostic and management questions related to the individual with a relevant history of a systemic reaction, but appropriate testing methods remain unable to detect any venom-specific IgE, perhaps it is really simple. Certainly as clinician scientists, we look at our testing methods, consider non-IgE-mediated possibilities including, but not limited to, occult mast cell disease. Perhaps, at least in some instances, we are overthinking the problem. Maybe we are not testing with the correct venom? There are many species of Hymenoptera capable of

inducing a systemic allergic reaction [8]. Although there is considerable cross-reactivity between closely related Hymenoptera, some species have unique antigens that do not cross-react. Commercially available extracts contain only a small portion of the potential stinging Hymenoptera. For example, there are 13 species of *Vespula* in North America; ALK and Hollister-Stier contain only 5 and 6, respectively. Similarly, there are 21 species of *Polistes*; ALK and Hollister-Stier contain only 5 and 3, respectively. Finally, though not especially aggressive, there are 46 species of *Bombus* with no available skin testing or treatment extract available in North America. Phadia immunoCAP® has testing capabilities of only a single *Bombus* species. Fortunately, currently available venom products both for skin and in vitro are seemingly effective in the majority of affected venom allergic individuals.

Duration of Treatment: How Long Is Long Enough?

As mentioned by Golden in Chap. 9, the package inserts recommend indefinite treatment with venom immunotherapy (VIT). However, clinicians' have struggled with this important and essential question of how long is long enough? As clinicians there is considerable variability on how we respond to this question as we balance the relative risk and benefits of treatment. Just as there are consequences of initiating a specific therapy there are consequences of not initiating, or in this case discontinuing a treatment. Unfortunately, as pointed out by Golden in Chap. 9, there has been no recent formal research on this matter. However, the consensus of experience globally seems to suggest that the 3–5-year duration should be sufficient in most individuals. This same body of experience has helped us to clarify who would benefit from lifelong therapy and who might be a candidate for a shorter period of time.

The decision to discontinue VIT or not is usually individualized with a cooperative understanding and decision-making process between the patient and the physician. High-risk individuals include individuals where the initial systemic reaction was especially severe such as a catastrophic airway compromise or cardiovascular collapse. These individuals may benefit from lifelong VIT treatment. Others at high risk would include a mast cell disease with elevated serum tryptase, systemic reactions during VIT treatment, insect species such as honeybee, other comorbidities, and other individual consideration such as quality of life, occupation, or recreational considerations.

On a final note, I often ponder about where we will be in this arena in coming years and what unanswered questions can be answered. Research funding is very limited and in some cases nonexistent. In recent years, some of the best work has originated in Europe, and they have done fine work. In North America, many of our most prolific researchers and teachers have passed. Indeed, Bob Riesman and John Yunginger, both prolific contributors to similar works in the past are gone, leaving David Golden and his group from John's Hopkins as the most persistent contributor to the field of insect allergy in North America. So the final, and perhaps the most important question is: Who will answer these unanswered question?

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Index

A
Acid phosphatase, 54
Active amines, 61
Aculeata, 17, 43–45
Acute kidney injury, 52
Acute renal failure (ARF), 167, 176, 177
Adverse reactions
  risk reduction, 133–136
  with skin testing, 125–126
  with VIT
    autoimmune complications, 132
    characterization, 127
    children and pregnancy, 133
    MCADs and baseline serum tryptase, 130–131
    medication, 131–132
    rush and ultra-rush immunotherapy, 129–130
    systemic allergic reactions, 127–129
Aerial yellow jacket, 29
Africanized honeybees (AHB), 34, 51, 168, 169
  allergic reactions *vs.* toxic reactions and venom components, 172–173
  avoidance, 179
  behavior-venom, 171, 172
  geographic spread, 170
  habitat suitability, 170, 171
  mortality, 178
  multiple and massive envenomation
    clinical presentation, evaluation and treatment, 174–178
    follow-up, 178
    potential direct treatment, 179
  sting site considerations, 173–174
African sleeping sickness, 258–259
African trypanosomiasis, 258–259
Allele-specific oligonucleotide-qPCR method, 290
Allergens, 48–50, 62, 86, 87, 193, 213–214
  Api m 1, 158–159
  Api m 2, 159
  Api m 3, 159
  Api m 4, 159–160
  Api m 5, 160
  Api m 7, 55, 56
  Api m 10, 160
American trypanosomiasis, 256–257
Anaphylaxis, 102
  aeroallergens, 210
  electrophoresis, 208
  hymenoptera allergy, 3, 4, 11
  hypersensitivity to mosquito bite, 209
  immune inflammatory disease, 209
  immunoblot, 208
  in vitro serologic immune inhibition technique, 208
  mosquito bite hypersensitivity, 208–210
  non-anaphylactic systemic symptoms, 210
  recurrent serum sickness-like reactions, 208
Angiotensin-converting enzyme inhibitors (ACEI), 73, 131–132, 146, 164–165
Anopheles spp., 249, 260, 261
  A. stephensi, 206
Ant, 14, 19–21, 47
  bullet, 23
  fire, 71, 248–249
  harvester, 22
  native fire, 190
  stinging, diagnosis of, 92–93
  velvet, 23
Antigen 5 (Ag5), 59, 94, 162
Apamin, 57, 173

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Apis spp. *See also* Honeybee
A. mellifera, 33–34, 44, 45, 59
A. mellifera scutellata, 34
Apocrita, 17
Arachnid, 238, 249, 254–256
Arthropod
adaptive immune responses, 231
allergic immune responses
delayed-type hypersensitivity reactions, 242–243
immediate-type hypersensitivity, 243
inflammation implications, 241
progression stages, 241
Arachnid, 238
arthropod-borne disease
alpha-gal allergy, 264
Arachnida, 249, 254–256
arthropod vector, 249–253
biological transmission, 248–249
characteristics, 248
geographic distribution, 247
Insecta, 256–264
mechanical transmission, 248
prevention and control, 263–264
red meat allergy, 264
skin injury mechanisms, 248
social stresses, 248
bedbugs, 239
centipede, 238
fleas, 239–240
head lice, 240
horseflies and deerflies, 236
human scabies mites, 240
indoor exposures
delayed-type inflammation, 231, 234
immediate effects, 231–233
immediate-type hypersensitivity, 233
innate responses, 231
late phase effects, 231, 233–234
morphology, 231, 232
neurotropic venom, 232–233
painful papules, causes, 234, 236
pruritic lesions, causes, 234, 235
saliva, toxic effects, 231
trauma, 231–232
lacewing larvae, 237
mosquito species, 235–236
sandfly bites, 237
skin infections, 237
thrips, 237
ticks, 239
true bugs, 237–238
Asian needle ant. *See Brachyponera chinensis*
Autoimmune diseases, 132

B
Babesiosis, 249, 254
Bald-faced hornet, 27
Baseline mast cell tryptase, 73, 114
Baseline serum tryptase concentration (BTC), 130–131, 288
Basophil activation test (BAT), 90–92, 163, 165
Basophil-based IgE antibody sensitization tests, 90–92
Basophil mediator (histamine) release (BHR) assay, 90
Beauveria bassiana, 55
Bedbugs, 239
Beekeepers, 168–169
Bee pollen, 167
Beeswax, 168
Beta-adrenergic blocking agents (BB), 146
Beta blockers, 73, 104, 131–132, 148
Black carpenter bees, 281
Bombus spp. *See also* Honeybee
B. impatiens, 36, 52
B. ternarius, 36
B. terrestris, 44
B. vosnesenskii, 36, 37
Bone marrow (BM) evaluation, 292
flow cytometric analysis, 292, 294
histology, mastocytosis, 292, 293
KIT mutation evaluation, 294
MC cytology, 292, 293
MC immunophenotyping, 292
smear morphology, 292
Brachyponera spp., 33
B. chinensis, 21, 22, 279
B. sennaarensis, 280
Bradykinin (BK), 58
Breakbone fever, 261
BTC. *See* Baseline serum tryptase concentration (BTC)
Bug-borne diseases, 256–257
Bullet ants, 23
Bumblebees, 35, 280–281. *See also Bombus* spp
nests, 46

C
CAP-FEIA. *See* Radioallergosorbent test (RAST)
Carboxylesterase, 56
CD marker
CD2, 294
CD25, 293
CD63, 90, 163
CD117, 293
CD203c, 90

Centipedes, 238
Chagas disease, 256–257
Claspers, 18
Clinical reactions
beta-blocker medications, 73
death, 75
honeybee allergy
honeybee-related products, 166–168
rare and unusual reactions, 169
sensitization/reaction, routes, 166
LLR, 72
observational multicenter study, 73
systemic reaction, 72
toxic reactions, 74
types of, 71
unusual reactions, 74
Clonal mast cell activation disorders (c-MCAD), 287, 295–298
Cold-induced urticaria, 102
Corticosteroids, 109, 234
Creatinine phosphokinase (CPK), 175
Cross-reactive carbohydrate determinants (CCD), 162

D
Deerflies, 236
Dengue fever, 261
Dipeptidyl peptidase IV (DPP IV), 55–56, 160
Diptera, 203
Dolichovespula spp., 25, 27
D. arenaria, 29
D. maculata, 27, 28
D. media, 45
nests of, 29

E
Ehrlichiosis, 254
Encephalitides
West Nile virus, 262–263
Zika virus, 263
Envenomation, 51, 52, 103
AHB
clinical presentation, evaluation, and treatment, 174–178
follow-up, 178
potential direct treatment, 179
Epidemic typhus, 259–260
Epinephrine, 104, 109, 110, 131, 135, 192
European fire ant. *See Myrmica rubra*
European honeybees (EHB), 170, 171
Exchange transfusion, 176

F
Female hard ticks, 239
Fire ants. *See Solenopsis* spp.
Flea-borne diseases
murine typhus, 257
plague, 258
Fleas, 239–240
Fly-borne diseases
African sleeping sickness, 258–259
leishmaniasis, 259
Formica, 19
Formicidae, 18–19, 47, 95
Formicine ants, 19

G
Gene Ontology (GO) terms, 60

H
Harvester ants. *See Pogonomyrmex*
Head lice, 240
Heterogeneity, 60–61, 242
Heteroptera, 233, 237–239
Histamine, 6, 12, 13, 58, 61, 220
Honey, 167
Honeybee, 45–46, 275–276
Apis mellifera, 33–34
Bombus, 35
B. impatiens, 36
B. ternarius, 36
B. vosnesenskii, 36, 37
genera of, 33
perennial colonies, 34
production, 34
species of, 33
toxicity, 51–53
Xylocopa, 37
Honeybee allergy, 118–119, 155, 156
AHB, 169
allergic reactions *vs.* toxic reactions and venom components, 172–173
avoidance, 179
behavior-venom, 171, 172
clinical presentation, evaluation, and treatment, 174–178
follow-up, multiple and massive envenomation, 178
habitat of, 170
mortality, 178
potential direct treatment, multiple and massive envenomation, 179
sting site considerations, 173–174

Honeybee allergy (*cont.*)
clinical reactions
honeybee-related products, 166–168
rare and unusual reactions, 169
sensitization/reaction, routes, 166
diagnosis, 161–163
entomological factors, 157
epidemiology, 156
stinger apparatus and sting mechanism, 157–158
treatment, 163–166
venom composition and relevance, 158–160
Honey pots, 35
Hornets, 27, 54, 59, 278
Horseflies, 208, 231, 232, 236
Human disease, arthropods and. *See* Arthropod-borne disease
Human scabies mites, 240
Hyaluronidase, 54, 62, 159, 172–173
Hymenoptera venom allergy (HVA), 95, 286, 308–309
allergens, 48–50
anaphylaxis, 3, 4, 11, 72, 73
ants, 14
clinical reactions
beta-blocker medications, 73
death, 75
LLR, 72
observational multicenter study, 73
systemic reaction, 72
toxic reactions, 74
types of, 71
unusual reactions, 74
compounds, biological function, 48–50, 53
active amines, 61
enzymes, 53–56
heterogeneity, 60–61
peptides, 56–59
protease inhibitors, 56
proteins with unknown function, 59–60
trace compounds, 60
cross reactivity patterns, 59
cutaneous allergic reactions, 112
discovery, era of, 4–5
emergency epinephrine, 110
IgE response, 75
immunotherapy
dose of, 78
immunological changes, 76
methods of, 76–77
mitigating factors, 76
reactions, 79–80
recommendations, 76
schedules, 77–78
side effects, 79
WBE, VIT and placebo, 75–76
mast cell diseases, screening indications, 297–298
mastocytosis
patient management, 300–301
sting reactions severity, 299
VIT safety, 299–300
mixed vespid venom, 115
origins of, 3
pathophysiology, 296–297
prevalence, 295–296
purified venom era, 12–13
standardization, 61–62
sting fatalities
adverse reactions, 101–102
anaphylaxis, causes of, 106
categories of, 102–103
death, Hymenoptera sting anaphylaxis, 106
incidence, 103
pathologic findings, 104–105
reactions, 105–106
risk factors, fatal sting anaphylaxis, 103–104
systemic allergic reactions, 111
toxicity of, 51–53
treatment, 109
venom apparatus, 43–44
vs. life cycle, 45–50
VIT, 110–111
honeybee allergy *vs.* vespid allergy, 118–119
injections, interval, 118
monitoring, 118
patient selection, 111–114
process, 116–117
safety of, 117
venom selection, 115–116
venom skin test-negative patient, 114
whole-body extract era
Archives of Internal Medicine, 6–7
Insect Allergy Committee of the American Academy of Allergy, 11–12
The Journal of Allergy, 5–6
Journal of the American Medical Association, 9–11
New England Journal of Medicine, 7–9
skin testing with, 11
uncertainty, 12
wasp venom allergy, 8
Hyposensitization, 7, 11

I
Icarapin, 160
Immulite system, 89, 94
ImmunoCAP system, 89, 90, 94
Immunotherapy, 7–9
  compliance with, 197–198
  dose of, 78
  dosing schedule, 192, 193
  efficacy, 194
  IFA
    dosing schedule, 192, 193
    efficacy, 194
    sensitization, skin testing, 197
  immunological changes, 76
  methods of, 76–77
  mitigating factors, 76
  mosquito bites, 221–222
  reactions, 79–80
  recommendations, 76
  RIT, 194–196
  schedules, 77–78
  side effects, 79
  VIT (*see* (Venom immunotherapy (VIT)))
  WBE and placebo, 75–76
Imported fire ant (IFA)
  allergen extract, 193
  characteristic sterile pseudopustule, 191
  cross-reactive antigens, 198
  economic damage, 189
  Hymenoptera, 190–191
  immunotherapy dosing schedule, 192, 193
  immunotherapy efficacy, 194
  morbidity and mortality, 192
  patient evaluation, 192
  prevention, 193–194
  RIT, 194–196
  skin testing, 196
  sting attack rates, 190
  systemic reactions, testing and treatment, 196–197
Insecta
  bug-borne diseases, 256–257
  flea-borne diseases, 257–258
  fly-borne diseases, 258–259
  Louse-borne diseases, 259–260
  mosquito-borne diseases, 260–264
Insect sting allergy
  behavior, 271–273
  death rates, 274
  diagnosis
    basophil-based IgE antibody sensitization tests, 90–92
    human contact, probability of, 92
    IgE antibody test with clinical history, 95
    molecular allergology, impact of, 93–95
    serological venom-specific IgE antibody analyses, 89–90
    skin tests, 86–88
    sting challenge provocation test, 88
    stinging Formicidae, 92–93
  ecology and distributions
    fire ants, 278–279
    harvester ants, 279
    honey bees, 275–276
    paper wasps, 277–278
    social wasps, 281
    solitary wasps, 281
    true hornets, 278
    yellowjackets, 276–277
  entomological aspects
    bees, 33–37
    Brachyponera chinensis, 21, 22
    bullet ants, 23
    female insects, 18
    fire ants, 19–20
    formicine ants, 19
    phylogeny, 17, 18
    Pogonomyrmex, 21, 22
    preventing stings, 38–40
    Vespidae (*see* (Vespidae))
  fear reduction, education, 274
  human behavior, 273–274
  medical treatment, 274
  quality of life, 273
  social structure, 272
Interleukin
  IL-3, 90
  IL-4, 75
  IL-13, 75
Intradermal-skin testing (ID-ST) technique, 88, 96
In vitro methods, insect allergy
  basophil-based IgE antibody sensitization tests, 90–92
  serological venom-specific IgE antibody analyses, 89–90
In vivo methods, insect allergy
  skin tests, 86–88
  sting challenge provocation test, 88

J
Jack jumper ant. *See Myrmecia pilosula*
Japanese hornet. *See Vespa crabro*

K
Killer bees. *See* Africanized honeybees (AHB)
Kinins, 58, 72, 86
Kounis syndrome, 169, 176
Kunitztype trypsin inhibitor, 56

L
Large local reaction (LLR), 10, 11, 61, 72, 101, 113, 126, 191, 203, 205, 220, 239
Leishmaniasis, 259
Lethal dose (LD50), 52
Lice, 240
Light-colored desert fire ant. *See Solenopsis aurea*
Little fire ant. *See Wasmannia auropunctata*
Louse-borne diseases, 259–260
Lyme disease, 254–255

M
Major royal jelly proteins (MRJPs), 60
Malaria, 209, 249, 260, 261
Mast cell activation disorders (MCADs), 130–131, 286, 287
Mast cell degranulating peptide (MCDP), 57
Mast cell (MC) diseases, 164, 308–309
  bone marrow evaluation, 292–294
  clonal mast cells, 286
  D816V KIT mutation screening, peripheral blood, 290, 292
  primary
    diagnosis, 288–293
    mastocytosis (*see* (Mastocytosis))
    monoclonal mast cell activation syndrome, 287
  skin examination
    diffuse cutaneous infiltration, 289, 291
    positive Darier's sign, 289, 291
    urticaria pigmentosa, 289–291
Mastocytosis, 103–104, 145
  abnormal features, 286
  allele-specific oligonucleotide-qPCR method, 290
  clinical presentation, 295
  diagnostic criteria, 286, 287
  diffuse bone sclerosis, 288
  epidemiology, 295
  histopathology, 292, 293
  for HVA
    patient management, 300–301
    sting reactions severity, 299
    VIT safety, 299–300
  osteopenia, 295
  osteoporosis, 295
  osteosclerosis, 295
  WHO classification, 286–288
Mastoparan C, 58
MCADs. *See* Mast cell activation disorders (MCADs)
Melittin, 57, 159–161, 172–173
Mischocyttarus sp., 32
  M. flavitarsis, 32–33
Monoclonal mast cell activation syndrome, 287
Mosquito
  allergic reactions, 235–236
  anaphylactic reactions, 236
  hypersensitivity
    allergic reactions, 204–205
    anaphylaxis, 208–210
    clinical and immunologic features, 205–207
    delayed-type hypersensitivity skin test antigen, 206–207
    diagnosis, 214
    heat-sensitive passive transfer, 205–206
    home remedies, 220
    immune responses, 206–208
    immunopathologic mechanisms, 222
    immunotherapy, 221–222
    insect repellents, 218, 219
    large local reactions, 205–207
    late-phase reactions, 221
    management of, 214–216
    natural history, 214
    non-IgE-mediated mast cell activation, 206
    pharmacologic treatments, 222–223
    prevention, 216–219
    prophylactic non-sedating antihistamines, 220
    research and development, 222, 223
    risk factors, 216
    saliva allergens, 213–214
    and salivary proteins, 210, 212–213
    saliva-specific IgE, 206
    serologic testing, 216
    skin testing, 215
    stages, 207
    taxonomy and distribution, 211–212
    therapeutic approaches, 220–221
    treatment, 216–219
    whole-body extracts, 214, 215
  mosquito-borne diseases

dengue fever, 261
encephalitides, 262–263
malaria, 260, 261
yellow fever, 262
papular urticarial, etiology, 236
MRJPs. *See* Major royal jelly proteins (MRJPs)
Multiplex chip-based assays, 96
Myrmecia, 52–53, 280
M. pilosula, 14, 57
Myrmica rubra, 198, 279
Myrmicinae, 19, 198, 280

N
Nylanderia spp.
N. fulva, 198, 199
N. pubens, 198

O
Omalizumab, 80, 134, 166
Ovipositor, 45

P
Pachycondyla spp., 52–53
P. chinensis, 21
P. goeldii, 57
Panel testing, 96
Paper wasp, 31, 32, 59, 277–278
Papular urticaria, 235–236, 242
Paraponera spp., 52–53
P. clavata (*see* (Bullet ants))
Paravespula, 24
Paulistine, 58
Pepsis sp., 23, 281
Peptides, 56–59
Phospholipase
Phospholipase A_1, 53
Phospholipase A_2, 53, 158–159, 172–173
Phospholipase B, 54
Plasmapheresis, 176
Platelet-activating factor (PAF), 105
Platelet-derived growth factor 1 (PVF1), 61
Pogonomyrmex spp., 21, 22, 279–280
P. badius, 53
Polistes spp., 31
P. dominula, 31, 32
P. dominulus, 44, 55
P. exclamans, 31, 32, 55
P. fuscatus, 31
Prausnitz-Kustner technique, 7
Primary mast cell diseases
diagnosis, 288–293
mastocytosis, 286–288
monoclonal mast cell activation syndrome, 287
Prophenoloxidase (proPO)-activating factor (PPAF), 55
Propolis, 166, 167
Protease inhibitors, 56
Proteins, 6, 24, 47, 54, 59–61, 92, 212, 213, 215
Provespa sp., 23

R
Radioallergosorbent test (RAST), 161–163
Red wasp, 31
Reverse transcription polymerase chain reaction (RT-PCR), 294
Rhabdomyolysis, 51, 106, 176
Rocky Mountain spotted fever, 255–256
Royal jelly, 166, 167
Rush immunotherapy (RIT), 8, 76, 78, 194–196

S
Salivary gland extract (SGE)
allergens, *Aedes aegypti* saliva, 213–214
biologic activity, 212
bite testing, 216
bronchial alveolar lavage, 212
diagnostic testing, 214
enzyme-linked immunosorbent assay, 215–216
glycerinated whole-body extract, 215
inflammatory bowel disease, 210
oral administration, 210
peripheral blood lymphocytes, 210
serologic testing, 216
skin testing, 215
therapeutics, 210
whole-body extracts, 214, 215
Samsum ant. *See Brachyponera sennaarensis*
Sand flies, 236–237
Sanger sequencing method, 294
Scabies, 240
Secapin, 58
Serine carboxypeptidase, 56
Serine proteases, 55
inhibitors, 56
Serological venom-specific IgE antibody analyses, 89–90

Serotonin, 61
Siemens Healthcare. *See* Immulite
Skin testing, 5, 86–88
  adverse reactions with, 125–126
  IFA, 196
  immunotherapy, IFA sensitization, 197
  mosquito bite hypersensitivity, 215
  SGE, mosquito, 215
  VIT, 309
SLIT. *See* Sublingual immunotherapy (SLIT)
Social stinging insects, 272–273
Social wasp, 281
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), 61, 192, 213
Solenopsis spp., 21, 45, 52–53, 278–279
  S. geminata, 21
  S. invicta, 14, 19, 20, 44, 198, 199
  S. richteri, 19, 20, 198
  S. xyloni, 21
Solitary wasp, 52, 281
Southern fire ant. *See Solenopsis xyloni*
Sting challenge provocation test, 88
Subcutaneous venom immunotherapy, 165
Sublingual immunotherapy (SLIT), 134
Sweat bees, 37, 281
Symphyta, 17
Systemic reaction, 72
  allergic, 111, 127–129, 133–136
  toxic, 103

T
Tabanidae, 236
Terebrantes, 17
Terebrantia, 43
Tetramorium bicarinatum, 61
Thermo Fisher Scientific. *See* ImmunoCAP
Thrips, 232, 237
Tick
  biting arthropods, 239
  insect-induced delayed-type reactions, 242
  tick-borne illnesses
    Babesiosis, 249, 254
    Ehrlichiosis, 254
    Lyme disease, 254–255
    Rocky Mountain spotted fever, 255–256
    Tularemia, 256
Toxic reactions, 74, 101
  AHB, 172–173
  clinical reactions, 74
  systemic reaction and, 103
Trace molecules, 60
Tropical fire ant. *See Solenopsis geminata*
True bugs, 237–238
True hornets, 26, 27, 29, 278
Tryptase, 103–105, 145
Tularemia, 256
2D gel separation, 60
Type 1 hypersensitivity reaction, 102

U
Unusual reactions
  honeybee stings, 69
  Hymenoptera stings, 74, 101–102

V
Venom apparatus
  evolution, 43–44
  and life cycle
    allergens, 48–50
    ants, 47
    bumblebee nests, 46–47
    honeybees, 45–46
    wasps, 47
Venom immunotherapy (VIT), 75–77, 110–111
  adverse reactions with
    autoimmune complications, 132
    characterization, 127
    children and pregnancy, 133
    MCADs and baseline serum tryptase, 130–131
    medication, 131–132
    rush and ultra-rush immunotherapy, 129–130
    systemic allergic reactions, 127–129
  basophil activation testing, 309
  duration of, 141–142
    immunologic changes/other test procedures, 144
    indefinite/extended treatment, candidates, 148
    individual/social factors, 146–147
    insect species, 145
    medical conditions and medications, 146
    pre-VIT sting reactions, history of, 144–145
    sting anaphylaxis, 142, 146
    systemic reaction, risk of, 142
    systemic reactions during, 145–146
    treatment, length of, 143

tryptase/mastocytosis, 145
efficacy, 300
honeybee allergy *vs.* vespid allergy, 118–119
injections, interval, 118
in vitro methods, 309
monitoring, 118
patient selection, 111–114
process, 116–117
recombinant methods, 309
safety, 117, 299–300
skin testing, 309
treatment, 310
venom selection, 115–116
venom skin test-negative patient, 114
Venomous animals, 43
Venom skin tests (VSTs), 111–112, 143, 144
Vespidae, 23, 44
bald-faced hornet, 27
Brachygastra spp., 33
Dolichovespula spp., 25, 27
D. arenaria, 29
D. maculata, 27, 28
nests of, 29
life cycles, 23
Mischocyttarus spp., 32–33
Polistes spp., 31
subfamilies, 23
Vespa spp., 29
V. crabro, 29–30
V. velutina, 30
Vespula spp., 24
V. germanica, 25, 26
V. pensylvanica, 25, 26
V. rufa, 26
V. squamosa, 26, 27
V. sulphurea, 26
V. vulgaris, 24–25, 30
VIT. *See* Venom immunotherapy (VIT)

W
Wasmannia auropunctata, 279
Wasps, 47. *See also* Vespidae
paper wasp, 31, 32, 59, 277–278
red wasp, 31
social wasp, 281
solitary wasp, 52, 281
West Nile fever syndrome, 262–263

X
Xylocopa spp., 37. *See also* Honeybee
X. virginica, 37

Y
Yellow fever, 262
Yellowjacket, 27, 133, 276–277. *See also* Wasps

Z
Zika virus, 263