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How Allergen Extracts Are Made—From Source Materials to Allergen Extracts

Allergenic extracts to diagnose and treat sensitivity to insect venoms and inhaled allergens

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ABSTRACT

Objective: To review allergenic extracts used to diagnose or treat insect allergies, including how the extracts are manufactured and their measurements of potency or concentration.
Data Sources: Peer-reviewed articles derived from searching PubMed (National Center for Biotechnology Information) about insect allergies and extract preparation. Encyclopedia of Life (http://www.eol.org/) and http://allergome.org/ were also referenced for background information on insects and associated allergens.
Study Selections: Search terms used for the PubMed searches included *insect allergens and allergies*, *Apidae*, *Vespidae*, *fire ants*, *cockroach allergies*, *insect allergen extract preparation*, and *standardization*.
Results: Humans may be sensitized to insect allergens by inhalation or through stings. Cockroaches and moths are predominantly responsible for inhalation insect allergy and are a major indoor allergen in urban settings. Bees, fire ants, and wasps are responsible for sting allergy. In the United States, there are multiple insect allergen products commercially available that are regulated by the US Food and Drug Administration. Of those extracts, honeybee venom and insect venom proteins are standardized with measurements of potency. The remaining insect allergen extracts are nonstandardized products that do not have potency measurements.
Conclusion: Sensitization to inhalational and stinging insect allergens is reported worldwide. Crude insect allergen extracts are used for diagnosis and specific immunotherapy. A variety of source materials are used by different manufacturers to prepare these extracts, which may result in qualitative differences that are not reflected in measurements of potency or protein concentration.

Introduction

Insects sensitize humans by 1 of 2 routes: inhalation or stings. The most common inhalational sensitizers are German and American cockroaches. Moths, butterflies, and silk worms may also sensitize by inhalation, but the most common offenders are cockroaches, which are major allergens in urban settings. Along with house dust mites, cockroaches may initiate the allergic march toward allergic asthma.[1]

Thousands of species of bees, wasps, and ants are capable of stinging when aggravated. Hymenoptera venom allergy refers to local or systemic allergic reactions in response to insect stings belonging to the order Hymenoptera. Hymenoptera populations vary by region. In Central and Western Europe, Hymenoptera venom allergy is primarily induced by yellow jacket or honeybee stings and less frequently by hornets or bumblebees. In parts of Southern Europe, paper wasp stings are most prevalent. In the United States, honeybees, wasps, and ants may induce allergic sensitization.[2]

Venom allergy after an insect sting is a classic example of cross-linked receptor–bound IgE antibodies that induce mast cell degranulation. Allergic reactions to venom may be localized to the site of the sting (swelling, redness, or itching) or systemic. Stings or bites from mosquitos, flies, ticks, and midges induce specific IgE but primarily cause local allergic reactions and therefore are not considered a major public health concern in allergic disease. By contrast, venoms from Apidae (honey and bumblebees), Vespidae (wasps such as yellow jackets, paper wasps, and hornets), and Formicidae (fire ants) are responsible for more than 10% of systemic allergic reactions and approximately 40 deaths in the United States each year.[3,4] Sting allergies may occur at any age but are less frequent in children than adults. Up to 5% of Apidae and Vespidae

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stings induce IgE-mediated hypersensitivity, and up to 32% of honeybee keepers are sensitized to honeybee venom.[2,5] We review the insects responsible for inhalational and venom allergy in the United States and elsewhere. We also review the extracts used to diagnose or treat venom allergy, including how they are manufactured and their measurements of potency or concentration.

Inhalational Insect Allergens

Cockroach

Inhalational allergy to cockroach was first reported in 1964.[6] There are more than 5,000 species of cockroaches that inhabit tropical forests and are not major inducers of inhalational allergy. By contrast, German and American cockroaches (*Blattella germanica* and *Periplaneta americana*, respectively) have adapted to cohabitate with humans and are the major source of perennial environmental indoor allergens in urban and low socioeconomic settings.[7,8] The prevalence of allergic sensitization to cockroach allergens in urban homes is high. More than 65% of all inner-city children and 35% of those who have asthma are allergic to cockroaches.[9]

Cockroach excretory products contain several allergens. In addition, body secretions and egg casings dry and become a component of airborne indoor dust that remain in the environment for several months after cockroaches are removed.[10,11] Unlike cat dander and ragweed pollen, there are no cockroach allergens that dominate the allergic response. Instead serum samples from cockroach allergic patients reveal complex patterns of reactivity to multiple proteins with no consistent patterns that typify allergic sensitization.[10] There are more than 10 groups of cockroach allergens (www.allergen.org) listed in the World Health Organization/International Union of Immunological Societies allergen database, but novel allergens have been identified in cockroach feces and whole bodies.[11–13] In addition to initiating an allergic response, proteolytic activity of some allergens may add to the severity of cockroach sensitization by disrupting the integrity of airway epithelial cells.[14,15]

A method of manufacturing cockroach allergen extracts starts with culturing cockroaches in a closed moist container that contains the insects and feed. The whole bodies, along with their secretions and excretions, are collected and killed by freezing. The killed frozen insect bodies serve as the source material for allergen extraction. After thawing, the bodies are milled to a fine powder, defatted with acetone, and air dried. The material is then mixed with an extraction buffer, which is usually glycerol and Coca solution or phosphate-buffered saline. The extraction is usually performed at 2°C to 8°C, after which the extracted material is centrifuged to remove solid particles.[7,16] Sometimes the bulk-extracted material is stored for few hours to days to allow precipitation of additional proteins before dispensing into vials for long-term storage. As may be inferred from its manufacturing process, cockroach extract is highly complex and may vary, depending on the chow fed to the roaches and the differences in the method of separation of roaches and their secretion and excretion products from the chow.

Cockroach allergen extracts are not standardized for potency, and therefore the finished vials are only labeled as weight/volume, which refers to the amount of source material in grams and the volume of extraction solution in milliliters. As is the case for all nonstandardized extracts, there are no controls for the composition and potency of cockroach extracts. The variability of protein concentration, fraction of allergenic proteins, and proportions of allergens differ among cockroach allergen extracts.[7] This variability among cockroach extracts and lack of standardization may contribute to the poor efficacy of cockroach allergen immunotherapy.[17] Complicating the complexity of cockroach allergen extracts is the presence of glycinin, a soybean allergen homolog, in a German cockroach fecal extract.[11] Rather than a component of the roaches, glycinin is likely a contaminant from the rodent chow fed to the cockroaches. If the extracts containing glycinin are used to diagnose cockroach allergy, then patients who are soybean sensitive may be incorrectly diagnosed as being allergic to cockroaches.

Silkworm Moth

The silkworm moth (*Bombyx mori*) is completely domesticated insect that cannot survive in the wild.[18] Silkworm moths are grown as sericultures for recovery of silk, and silkworm pupae are a popular traditional food in much of Southern and Eastern Asia. The moths are traditionally perceived as a source of occupational inhalant allergens in silk-producing industries. Silk and silk waste products used as filler in bed mattresses contain several IgE-binding allergens and are also responsible for contact allergies.[19] As many as 40% of patients with respiratory allergy in Guangzhou, Southern China, are sensitized to silkworm moth. Almost 50% of those patients are also sensitized to 1 of 9 additional inhalant allergens, including 3 species of house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia tropicalis*) and German and American cockroach.[20] In addition, those who are allergic to silkworm moths are often sensitive to other moths and butterflies.[21]

Compared with cockroach allergens, silkworm moth allergens are poorly defined. Arginine kinase (Bomb m 1, 42 kDa) has been identified as a major silkworm allergen and is cross-reactive to a similar cockroach allergen.[19,22] Multiple allergens with molecular weight ranging from 14 to 70 kDa are described in the components of silkworm moth waste, which is used as a mattress filler.[23] In 2016, Jeong and colleagues[24] identified a heat stable 27-kDa glycoprotein as an allergen in silkworm pupae extract that was boiled for 5 minutes.[24]

Crude allergen extracts of *Bombyx mori* are prepared from the wings covered with scales. Abdominal cuticular pieces can also be used as an allergen source. The collected material is cut into smaller pieces before maceration and defatting with ethyl ether or acetone. For allergen extraction, the powdered material is mixed with buffer solution (1:10 or 1:20 wt/vol ratio) and allowed to mix for 24 to 48 hours at 2°C to 8°C. The extract is then centrifuged and the supernatant filtered before use for skin testing.[25]

Stinging Insect Allergens

There are 3 families of stinging insects: Apidae (honeybee, bumblebee), Vespidae (yellow jacket, yellow hornet, white-faced hornet, paper wasp) and Formicidae (fire ant, harvester ant, jack jumper ant) (Table 1).

Honeybee Venoms

Wild honeybees (*Apis mellifera*) nest in tree hollows, rotted logs, and occasionally in voids in walls of homes and other buildings. Honeybees are generally not aggressive and sting only when they sense danger to the nest or the queen. IgE-mediated anaphylaxis to honeybee venom is one of the most severe forms of Hymenoptera venom allergy. Honeybee sting reactions range from local reactions to anaphylactic shock and death. As reviewed by Bilo and Bonifazi,[26] the prevalence of systemic reactions in adults to Hymenoptera stings ranges from 0.5% to 3.3% in the United States and 0.3% to 7.5% in Europe. Among those who are allergic to honeybee venom, 0.3% to 42.8% of systemic allergic reactions were considered life-threatening (ie, classified as anaphylaxis).

Although honeybee venom has historically been considered well characterized, 2 research groups used proteomic methods to identify more than 80 peptides or proteins in honeybee venom, of which 4 are proteins that are potentially novel allergens.[27,28] Among the 12 allergens that are well described, the 3 major honeybee venom allergens are phospholipase A2 (Api m 1),