

11 Cockroach and Other Inhalant Insect Allergens

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INTRODUCTION

Insect inhalant allergy is a health problem all over the world due to the cosmopolitan existence of these arthropods. The variety and distribution of insects and the accumulation of debris associated with heavy infestations vary significantly with time and geographic location. Insect inhalant allergens are found indoors, outdoors, in homes, and at the workplace. Sensitization can be due to involuntary exposure to windborne insect emanations in house dust or to occupational exposures, encountered by professionals such as research entomologists. The concept of inhalant insect allergies was developed by the early observations of Figley (1929), Parlato (1930, 1932), and Kern (1938) of asthma associated to sensitization to the mayfly, sandfly, mushroom fly, moth, and butterflies (1,2). In the animal kingdom, the phylum Arthropoda constitutes 75% of known animal species that can contribute significant organic material for airborne dispersal. Three major taxonomic groups, Insecta, Crustacea, and Arachnida, are of major concern as allergen producers. Some of the allergens from the three groups share amino acid sequence homology that manifests in allergenic cross-reactivity.

This chapter focuses on the class Insecta, which includes more than 80% of species from the three groups combined. Insects have three pairs of legs and bodies divided into head, thorax, and abdomen, and they are either wingless or have one or two pairs of wings. Cockroaches, mayflies, caddisflies, moths, butterflies, flies, fleas, midges, ants, bees, and vespids are representative members of this class. Insect allergy (i.e., IgE-mediated sensitivity) may be induced by a wide variety of insect-derived allergens in the environment either on a seasonal (vast aquatic insect emergences, such as caddisflies, mayflies, and midges) or perennial basis (terrestrial pests, such as cockroaches). In certain places, inhalant insect dust is clearly visible and associated with the emergence of caddisflies in May, June, and July. In Japan, documented sensitization to moths and butterflies is as common as sensitization to house-dust mite. Chironomidae larvae and midges cause allergic reactions in approximately 20% of environmentally exposed workers with insect larvae and subjects living in affected areas. Exposure to large numbers of the "green nimitti" midge in Sudanese communities is associated with an increased incidence of both asthma and allergic rhinitis. Honeybees produce "bee dust" that causes inhalant allergy in beekeepers, and subjects extracting bee venom can develop inhalant allergy to phospholipase C. Wherever allergenic exposure (onset, intensity, and frequency) and adjuvant forces (ozone, NO₂, tobacco smoke, viruses, etc.) are present in the environment, allergic symptoms can develop, particularly in those with a genetic atopic predisposition.

Cockroach allergy is especially important for the development of asthma in inner cities among lower socioeconomic groups (3-7). Over 4000 species of cockroach are described worldwide, the majority of which are not directly associated with humans in their home and work environments. Cockroaches can be categorized as domestic, peridomestic, or feral. Feral species are those that survive independent of humans and represent 95% of all species worldwide. Seventy-four species occur in the United States, some of which have been introduced from other parts of the world. Domestic species include the German and brown-banded cockroaches that live almost exclusively indoors and depend on human refuse (harborage food and water) for survival. Their ideal environment is warm and humid, making indoor households primary dwelling places; however, some species live outdoors. Peridomestic species include those that survive in or around domestic environments. This group is represented by American, Australian, brown and smoky brown, Oriental, and woods cockroaches. These were introduced into North America over the past two centuries and have been very successful in establishing habitats throughout the world including industrialized/highly developed countries where insect infestation is better controlled.

The desire to control the indoor climate with air conditioning units to mitigate extremes of temperature, moisture, and airflow sets the stage for several cockroach species to infest and inhabit homes. The presence of some domestic species in dwellings, such as the German or brown-banded cockroaches, is often a sign of poor sanitation or substandard housekeeping. Survival of these species is enhanced by crowded living, as in apartment complexes, where associated clutter and accumulation of organic debris is often present. An overpopulation of the peridomestic American or Oriental cockroaches in their native habitats, such as municipal sewage systems and septic tank areas, facilitates their entrance into nearby homes through crawl spaces, construction joints, and attic vents, causing infestation of even the best kept homes and workplaces. The species that infest household structures typically have a high reproduction potential, which results in accumulation of relatively high dust levels of cockroach airborne allergenic proteins derived from shed exoskeletons (cast skins) and feces.

ORIGIN AND TAXONOMY OF COCKROACHES

Some fossils attributed to cockroaches date back to the Carboniferous period from the Paleozoic era (over 350 million years ago), representing some of the oldest and most primitive of insects. However, such an early origin of cockroaches cannot be dated with reasonable confidence, and the idea of an ancient origin of cockroaches is been revised. The only obvious character in these fossils (a long ovipositor) is inconsistent with an hypothesis of relationship with cockroaches, and the first fossils of "modern" cockroaches with short ovipositors appear later, in the early Cretaceous, Mesozoic era, like most other insects (8).

Cockroaches belong to the phylum Arthropoda, class Insecta, and there are six cockroach families in the order Blattaria: Blaberidae, Blattellidae, Blattidae, Cryptocercidae, Nocticolidae, and Polyphagidae. The second and third families contain the most common peridomestic pests found throughout the world (Table 1). A more detailed taxonomy of cockroaches can be found in the publications by Atkinson and Koehler (9,10).

COCKROACH IDENTIFICATION

Cockroaches have an exoskeleton, a segmented body (head, thorax, and abdomen), three pairs of legs, and one or two pairs of wings or none. The possession of an exoskeleton gives the insect its form, attachment points for muscles, and provides a hardened protective covering (exoskeleton) that requires molting for growth. The old exoskeleton is discarded as exuviae (cast skins), allowing the insect to enlarge before a new exoskeleton hardens. Cockroaches are omnivorous and will consume any organic material, including fresh and processed foods, stored products, and even bookbindings and paste found on stamps and in wallpaper. In times of food shortage, some species will become cannibalistic to maintain a colony.

Table 1 Taxonomy of Cockroaches

Phylum:	Arthropoda	
Class:	Insecta	
Order:	Blattaria	
Family	Genus/species	Common name
Blaberidae	<i>Leucophaea maderae</i>	Madeira
Blattellidae	<i>Blattella germanica</i>	German
	<i>Blattella asahinai</i>	Asian
Blattidae	<i>Supella longipalpa</i>	Brown-banded
	<i>Periplaneta americana</i>	American
	<i>Periplaneta australasiae</i>	Australian
	<i>Periplaneta brunnea</i>	Brown
	<i>Periplaneta fuliginosa</i>	Smoky brown
	<i>Blatta orientalis</i>	Oriental

Table 2

Name

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Asian
Blattella

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Table 2 Cockroach Identification

Name	Morphology	Features	Habitat
German <i>Blattella germanica</i>	16 mm long, brown, parallel dark bands along axis of body	Incapable of flight, nocturnal. Varying degrees of pesticide resistance. Most prominent pest. Strictly domestic	Kitchens, pantries, bathrooms, bedrooms
Asian <i>Blattella asahinai</i>	16 mm long, light brown, requires taxonomist for differentiation from German	Capable of flight, attracted to light. Wild and peridomestic. Introduced in Tampa and Lakeland, Florida, 1986. Interbreed with German	Rich ground cover, citrus grooves of Florida leaf litter; manicured lawns
American <i>Periplaneta americana</i>	34–53 mm long reddish brown with variation light	Capable of flight; mostly cosmopolitan. Peridomestic	Landfills, crawlspaces, sewage systems, storm drains, septic tanks, attics, and dark tree holes, caves, mines
Oriental <i>Blatta orientalis</i>	25 to 35 mm long, black or dark brown	May or may not fly. Commonly known as water bug	Dark, damp conditions, water meter boxes, garbage chutes
Somky brown <i>Periplaneta fuliginosa</i>	25 to 33 mm long, dark brown	Major southern U.S. pest. Peridomestic, majority are wild	Tree holes, palm trees, loose mulch (pine bark, straw), firewood piles, soffits, panel walls, block wall interstices, false ceilings
Brown-banded <i>Supella longipalpa</i>	13–14.5 mm long, dark band across abdomen	Capable of flight, attracted to light	Nonfood areas, bedrooms, closets, living rooms

Infestations of cockroaches in primary dwellings and workplaces represent one of the most intimate and chronic associations of pests with humans. All cockroach species are adept crawlers; however, flight ability varies. The two most common species are the American (*Periplaneta americana*) and German (*Blattella germanica*) cockroaches. Table 2 describes the five major cockroach species associated with humans and their immediate environment.

PUBLIC HEALTH IMPORTANCE OF COCKROACHES

Cockroaches may adversely affect human health in several ways through biting, psychological stress and contamination of food with excrement (causing vomiting and diarrhea), associated pathogens, and allergy. Bernton and Brown made the first reports of cockroach sensitization in the 1960s (1,11). Kang and Sulit showed that 60% of patients with asthma in the Chicago area had positive skin tests, serum IgE antibodies, or positive bronchial challenge tests to *B. germanica* allergens (12). The National Cooperative Inner-City Asthma Study found that exposure and sensitization to cockroach allergens were associated with asthma morbidity in children from eight major inner-city areas in the United States. Of 476 children with asthma (age 4–7 yr), 36.8% were allergic to cockroach allergens, followed by dust mite (34.9%) and cat (22.7%) (6). The same association was found in children for cockroaches, and not for mite, cat and dog, in another Inner-City Asthma Study, reporting a 69% prevalence of sensitization to cockroach allergens in 937 children, reaching the highest value of 81% in the Bronx (7). Other parts of the world have reported an important association between cockroach infestations and asthma, including occupational asthma. Infestations by domiciliary cockroaches are largely dependent on housing conditions, and high-rise apartments have higher levels of cockroach allergens (7,13,14).

Americans now spend more than 95% of their time indoors in homes that are better insulated and temperature controlled while outdoor air exchange has been drastically reduced, creating conditions that support both pest growth and associated dust accumulation in the

home (6,7,11,12,15,16). Cockroach allergy can result from initial sensitization to the allergen though inhalation, ingestion, dermal abrasion, or injection. Potential sources of relevant cockroach allergens in the environment include whole bodies, cast skins, secretions, egg casings, and/or fecal material. Airborne cockroach allergens are associated primarily with amorphous and larger particles (10 μm) (which settle after disturbance) than are animal allergens. The threshold levels of allergen exposure above which susceptible individuals are at increased risk for sensitization or asthma symptoms are 2 and 8 U/g of dust for Bla g 1 or Bla g 2 (equivalent to 80 and 160 ng/g for Bla g 2) (4). In a nationally representative sample, cockroach allergen (Bla g 1) concentrations exceeded 2 U/g in 11% of U.S. living room floors and 13% of kitchen floors, and exceeded 8 U/g in 3% of living room floors and 10% of kitchen floors. Detectable concentrations of >0.4 U/g were found in 27.4% of U.S. homes (14). Mild-to-moderate symptoms induced by cockroach allergen inhalation include sneezing and rhinorrhea, skin reactions (mild dermatitis), and eye irritation, with difficulty in breathing and anaphylactic episodes occurring in more severely allergic individuals.

EARLY IDENTIFICATION OF COCKROACH ALLERGENS AND ASSESSMENT OF ENVIRONMENTAL EXPOSURE

Several groups used conventional physicochemical techniques to identify and characterize cockroach allergens. Allergenic proteins, with molecular weights ranging from 6 to 120 kDa, were initially identified from cockroach extracts using serum IgE from cockroach-sensitive individuals (17–19). A wide variation in their IgE-binding patterns to extracts of crude whole-body German cockroaches was observed. A high correlation of radioallergosorbent test (RAST) activity between German whole-body and fecal extracts was observed, and five allergens (MWs of 67, 60, 50, 45, and 36 kDa) showed IgE-binding reactivity in 50% to 80% of 37 human sera tested (19). Twarog et al. found three major allergens with molecular weights of 25, 63 to 65, and <10 kDa (20). Helm et al. identified a 36-kDa protein as a principal allergen of German cockroach whole-body extracts, not present in extracts of armyworm, caddisfly, lakefly, and other insects, and a 55-kDa protein also present in true armyworm, honeybee, and lakefly extracts (21). Crude extracts of whole-body American cockroach contain at least 29 antigenic components from which 18 were allergens (22,23). Two of these are major allergens (78 and 72 kDa) and cause T-cell proliferation of peripheral blood cells from cockroach-allergic patients (24). Zwick et al. localized, by immunohistochemistry, proteins derived from the epithelial cells of the intestinal tract that are present in the feces as well as in whole-body sections. They could represent important cockroach allergens (25).

Visual assessment of cockroach infestations correlates with skin test results. However, the best way to assess environmental concentration of cockroach allergens is by using enzyme-linked immunoassays. Two allergens from the German cockroach, Bla g 1 and Bla g 2, were purified using monoclonal antibodies (26). Bla g 1 is a 25 kDa acidic, cross-reacting allergen previously identified by Twarog et al., and Bla g 2, a 36 kDa species-specific allergen (20). Specific immunoassays for both allergens are used to monitor environmental cockroach exposure (26–28). A sandwich ELISA, based on a monospecific rabbit antibody reactive with Per a 1 and Bla g 1, had also been suggested for use in environmental assays (28). Other monoclonal antibodies to American cockroach allergens have also been produced that could be used to measure allergen levels (23,29).

MOLECULAR STRUCTURE AND BIOLOGICAL FUNCTION OF COCKROACH ALLERGENS

In the last 12 years, several cockroach allergens have been cloned, and their biochemical activities and biological roles investigated. American and German cockroach cDNA expression libraries have been screened with human IgE or murine monoclonal antibodies to identify clones expressing allergens. A list of the cockroach allergens reported by the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Subcommittee is shown in Table 3.

Table 3 Nom

Allergen

<i>B. germanica</i>
Bla g 1.010
Bla g 1.010
Bla g 1.020
Bla g 2 ^b
Bla g 4
Bla g 5
Bla g 6.010
Bla g 6.020
Bla g 6.030
Bla g 7
Bla g 8
<i>P. americana</i>
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Table 3 Nomenclature and Function of Cockroach Allergens (WHO/IUIS)

Allergen	MW ^a	Function/homology (kDa)	Accession number	References
<i>B. germanica</i>				
Bla g 1.0101	46,21	Midgut microvilli protein-homolog	AF072219, AF072221	31
Bla g 1.0102	90	Midgut microvilli protein-homolog	L47595	30
Bla g 1.0201	56	Midgut microvilli protein-homolog	AF072220	31
Bla g 2 ^b	36	Unusual aspartic protease	U28863	37,39,42
Bla g 4	21	Lipocalin	U40767	44
Bla g 5	23	Glutathione S-Transferase	U92412	50
Bla g 6.0101	17	Troponin C	DQ279092	51
Bla g 6.0201	17	Troponin C	DQ279093	51
Bla g 6.0301	17	Troponin C	DQ279094	51
Bla g 7	33	Tropomyosin	AF260897	54
Bla g 8	21	Myosin light chain	DQ389157	51
<i>P. americana</i>				
Per a 1.0101	26	Midgut microvilli protein-homolog	AF072222	32
Per a 1.0102	26	Midgut microvilli protein-homolog	U78970	33
Per a 1.0103	45	Midgut microvilli protein-homolog	U69957	104
Per a 1.0104	31	Midgut microvilli protein-homolog	U69261	33
Per a 1.0201	51	Midgut microvilli protein-homolog	U69260	105
Per a 3.0101	79	Arylphorin/hemocyanin	L40818	56
Per a 3.0201	75	Arylphorin/hemocyanin	L40820	56
Per a 3.0202	56	Arylphorin/hemocyanin	L40819	57
Per a 3.0203	46	Arylphorin/hemocyanin	L40821	57
Per a 6	17	Troponin C	AY792950	51
Per a 7.0101	33	Tropomyosin	Y14854	52
Per a 7.0102	33	Tropomyosin	AF106961	53
Per a 9	43	Arginine kinase	AY563004	72
Per a 10	28	Serine protease	—	—

^aMolecular weight calculated from amino acid sequence.^bThe X-ray crystal structure of Bla g 2 is accessible in the Protein Data Bank under the accession number 1Y99.

Helm et al. identified the first Bla g 1 clone that contains a 4 kb insert encoding for a protein with an apparent molecular weight of 90 kDa (Bla g 90 kDa or Bla g 1.0102) (30). The DNA encoding for Bla g 90 kDa consists of seven 576 bp tandem repeats with a shorter unique region at both ends. Other Bla g 1 and Per a 1 isoforms were identified in subsequent studies (31–33). Each of the tandem nucleotide repeats encodes for two consecutive amino acid repeats of approximately 100 residues each. DNA sequence analysis shows that Group 1 cockroach allergens originated by gene duplication and subsequent mutagenesis of a mitochondrial energy transfer domain (31). These allergens are thought to be involved in digestion, although the specific function is unknown. Bla g 1 is most prevalent in the midgut, and the Bla g 1 gene is exclusively expressed in midgut cells (31,34). Adult females produce and excrete significantly more Bla g 1 in their feces than males and nymphs, most likely because females process more food than males and other life stages of the cockroach, and production is related to food intake (35).

Bla g 2 is the most important among other cockroach allergens (rBla g 1, rBla g 4, rBla g 5, rPer a 7) (36) and has the highest prevalence of IgE antibody binding (54.4%), which is approximately twofold higher than the prevalence to any of the other four allergens tested using 118 sera from cockroach-sensitized subjects. Among sera with high IgE antibody levels to cockroach extract (3.5–100 IU/mL), the prevalence of IgE antibodies to Bla g 2 and Bla g 5 is 71% and 58%, respectively (36). These results confirm previous findings that Bla g 2 elicits IgE responses in 42% to 70% of cockroach-allergic patients. The prevalence of IgE antibody binding to Bla g 1 and Bla g 2 is 30% and 58%, respectively, using 106 sera from cockroach-allergic patients (37). Bla g 2 is homologous to aspartic proteases, which are a widely distributed group of digestive enzymes. Several studies support the idea that allergens with proteolytic activity may achieve access to antigen-presenting cells in the absence of inflammation by damaging the epithelium and facilitating their own access and penetration into the mucosa (38). For example, proteolytic activity of mite allergens (Der p 1, Der p 3, Der p 6) may contribute to allergenicity. However, Bla g 2 is a proteolytically inactive and potent allergen, inducing sensitization at

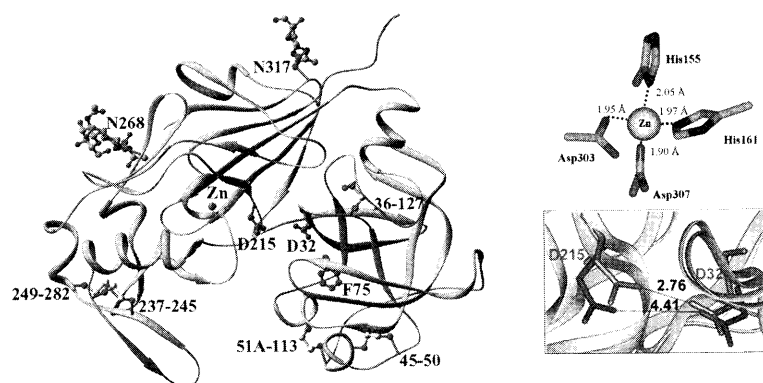


Figure 1 (See color insert.) Crystal structure of the cockroach allergen Bla g 2. (Left) Bilobal structure of Bla g 2 showing residues at the region of the catalytic site (D215, D32) and F75 (previous to insertion F75a), carbohydrates and the five disulfide bonds (1yg9.pdb). (Right) The zinc ion (sphere) with coordinating residues and interatomic distances (top); and aspartate positions in Bla g 2 (dark grey aspartates) and in pepsin (light grey aspartates) (bottom). Source: Reprinted with permission from Ref. 39.

exposure levels that are one or two orders of magnitude lower than other allergens such as Der p 1 (39,40).

The X-ray crystallographic structure of Bla g 2 has been solved, revealing interesting features that contribute to allergenicity and explain why Bla g 2 is not an active aspartic protease (Fig. 1, in color insert) (39). Aspartic proteases have a bilobal molecular structure, and the catalytic activity depends on a couple of amino acid triads (DTG) at the bottom of a cleft between the two lobes. However, Bla g 2 has important amino acid substitutions in the catalytic site, especially at the level of the triads (DST and DTS instead of DTG) that make this molecule enzymatically inactive (41,42). Distortions in the catalytic site, due to these substitutions, lead to a bigger distance of the catalytic aspartates, which is expected to impair enzymatic activity (Fig. 1, bottom right). In addition, there is an insertion (F75a) that interferes with the typical substrate-binding site, providing an autoinhibitory mechanism of enzymatic activity (39). These structural studies indicate that proteolytic activity is not necessary for allergenicity (41–43).

Two unique structural characteristics of Bla g 2 may contribute to allergenicity by conferring stability to the molecule: (i) the allergen binds one atom of zinc (Fig. 1, top right) and (ii) Bla g 2 has five disulfide bonds in contrast to only two or three in active aspartic proteases (Fig. 1, left) (39). Chronic exposure to low doses (1–10 µg/yr) of this stable cockroach allergen may explain why sensitization and exposure to Bla g 2 is associated with asthma.

Bla g 4 is another *B. germanica* allergen that belongs to the family of proteins called lipocalins (44). Apart from another insect lipocalin allergen identified and cloned from the biting reduviid *Triatoma protracta*, most of the known lipocalin allergens are from mammalian origin: Bos d 2 (cow), Can f 1 and Can f 2 (dog), Equ c 1 (horse), Fel d 4 (cat), Mus m 1 (mouse) and Rat n 1 (rat) (38,45,46). The milk allergen β -lactoglobulin (Bos d 5) is also a lipocalin. The molecular structure of these allergens is very stable and consists of a C-terminal α -helix and a β -barrel enclosing an internal hydrophobic cavity that binds small ligands such as retinoids, glucocorticosteroids, and pheromones (Fig. 2) (47). The homology of Bla g 4 (calycin) with rodent urinary proteins raises the possibility of pheromone or lipid-transport proteins being potential families of inhalant arthropod allergens. Immunohistochemical localization studies show that Bla g 4 is only expressed in the accessory glands of the male cockroach reproductive system (conglobate gland and utricles) and is transferred to the female during copulation. The study suggests that Bla g 4 has a reproductive function, and the allergen could be released from dried seminal secretions or spermatophores into the environment (48).

Additional cloned allergens are the glutathione S-transferase (Bla g 5), which could have a detoxifying function, and two proteins involved in muscle contraction: troponin C (Bla g 6) and tropomyosin (Group 7) (49–54). Troponin C has a calcium-dependent regulatory function, and calcium binding induces changes in the molecular structure that affect IgE antibody binding (51). Troponins and tropomyosins include a diverse group of proteins with distinct

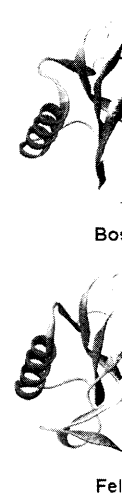


Figure 2 (cow), Equ c 1 (horse) obtained using X-ray crystallography of the molecule.

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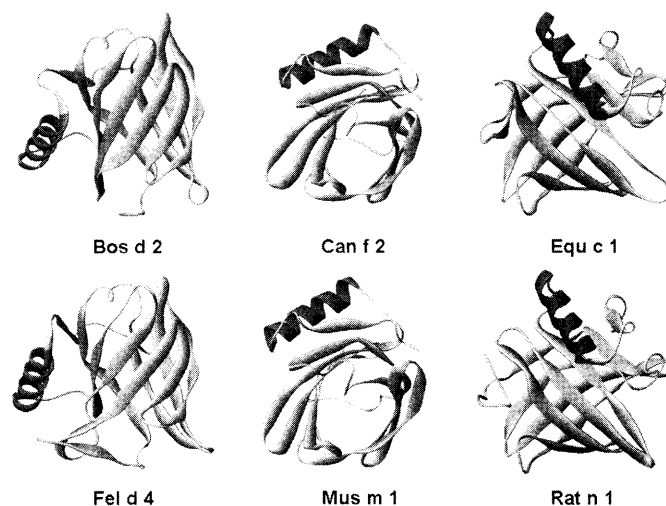


Figure 2 Ribbon representation of the molecular structure of Bla g 4 homolog allergens from mammals: Bos d 2 (cow), Equ c 1 (horse), Mus m 1 (mouse), and Rat n 1 (rat), and molecular models of Can f 1 (dog) and Fel d 4 (cat) obtained using Swiss-Model (106–108). The C-terminal α -helix is shown in dark gray. Three different orientations of the molecules are shown.

isoforms found in muscle, brain, and non-muscle tissue. Structurally, tropomyosins are elongated two-stranded proteins wound around each other with dimeric α -helical coiled structures along their length (55). The considerable homology of tropomyosins from different species explains part of the allergenic cross-reactivity among arthropods and mollusks (section Extra Species Cross-Reactivity).

Per a 3 shows high homology to insect hemolymph proteins (56). Isoallergenic variants Per a 3.0202 (C13) and Per a 3.0203 (C28) of the allergen Per a 3.0201 (C20) display significant differences in skin reactivity, suggesting a high degree of polymorphism among the allergens (57). Hemoglobins of the Diptera (insect) family of Chironomidae are causative agents in asthmatic patients living in regions where large swarms of nonbiting midges occur. Chi t 1, the hemoglobin from the European midge species (*Chironomus thummi*), represents the major allergenic component causing rhinitis, conjunctivitis, and bronchial asthma in exposed populations. There is considerable immunological cross-reactivity between hemoglobins of the same and closely related Chironomidae species, suggesting that hemoglobins and hemocyanins of insects may also represent an important source of arthropod allergens (58).

OTHER SOURCES OF INSECT ALLERGENS

Arthropods that have been most studied as sources of allergens include crustaceans (mussels, snails, squids), insects (caddisflies or sedges, mayflies, moths and butterflies, chironomid midges, and cockroaches), and arachnids (mites). A number of other arthropods, including the houseflies, ants, spiders, locusts and grasshoppers, bees and silverfish (in this case it is a single genus, species; each of the other groups consists of several known species producing allergens), cause sensitization either in the home or occupational settings. Table 4 shows a summary of insect species and their association to inhalant allergies.

The role of insects as providing inhalant allergens is further supported by data showing positive bronchial or nasal challenge. Airborne insect-derived particles include shed hairs, scales, excreta, and disintegrated body parts that contribute to amorphous dust. The composition of dust is influenced by geographical location, diligence and thoroughness of cleaning, use of insecticides, and both qualitative and quantitative sampling. The widespread incidence of swarming insects outdoors and the presence of cockroaches in house-dust samples and their related allergenicity are firmly established. Less certainty exists for other insect allergens serving as allergen source material in dust samples.

Dogs and cats contribute dander, hair, and body secretions to allergenic loads in household dust. Not widely known is the contribution of the common flea. When dogs and

Table 4 Inhalant Insect Allergies

Species	Common name	Order	Place of exposure	
<i>Apis mellifera</i>	Honeybee	Hymenoptera	Environment of beekeepers (O)	Rudeschko, 2004; Bousquet, 1982
<i>Bombyx mori</i>	Silkworm	Lepidoptera	Bedding, clothes; silk workers (O)	Kino, 1987; Wen, 1990; Uragoda, 1991; Suzuki, 1995; Komase, 1997; Hirabayashi, 1997
<i>Chironomus</i> sp.	Midges, chironomids	Diptera	Hypereutrophic lake in Japan Outdoors	Kino, 1987; Eriksson, 1989 Baur, 1992; Liebers, 1993; Ree, 1996; Komase, 1997; Hirabayashi, 1997; Yong, 1999; Morsy, 2000
<i>Ctenocephalides felis felis</i>	Cat flea	Siphonaptera	House dust	Trudeau, 1993
<i>Ephestia kuehniella</i>	Flour moth	Lepidoptera	Bakers (O)	Makinen-Kiljunen, 2001
<i>Harmonia axyridis</i>	Asian lady beetle	Coleoptera	Infested homes	Albright, 2006; Goetz, 2007; Nakazawa, 2007
<i>Lepisma saccharina</i>	Silverfish	Zygentoma	Infested homes	Wittman, 1996; Barletta, 2002; Barletta, 2005
<i>Locusta migratoria</i>	Grasshopper	Orthoptera	Research laboratories (O)	Soparkar, 1993; Lopata, 2005
<i>Lucilia cuprina</i>	Blowfly	Diptera	Entomological research laboratory (O)	Kaufman, 1986
<i>Monomorium pharaonis</i>	Pharaoh ant	Hymenoptera	Indoor infested environments	Kim, 2005; Kim, 2007
<i>Musca domestica</i>	Common housefly	Diptera	Closed breeding rooms; livestock stables, barns; pharmaceutical industry; workers (O)	Wahl, 1997; Tas, 2007; Focke, 2003; Barletta, 2003; Smith, 2005
<i>Sitophilus oryzae</i>	Rice weevil	Coleoptera	Infested building	Kleine-Tabbe, 1992
<i>Tenebrio molitor</i>	Mealworm (beetle larvae)	Coleoptera	Warehouse fishing bait handling (O)	Bernstein, 1983; Siracusa, 1994
<i>Thaumetopoea pityocampa</i>	Pine processionary (caterpillar)	Lepidoptera	Airborne urticating hairs	Werno, 1993; Fuentes, 2006
<i>Triatoma infestans</i>	Reduviid	Hemiptera	Indoor and outdoor environments	Alonso, 1996
Several species	Caddisflies	Trichoptera	Outdoor environments; hydroelectric plant workers (O)	Kino, 1987; Koshte, 1989; Warrington, 2003; Smith, 2005
Several species	Mayflies	Ephemeroptera	Outdoor rural environment	Smith, 2005

Occupational exposures are indicated by (O). Usually housefly, caddisfly, and mayfly implies plural species. Studies are cited by first author and year of publication.

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cats are present in the house, the dog flea, *Ctenocephalides canis*, and the cat flea, *C. felis*, can reach pest proportions. Most flea allergenicity has been attributed to bites from these insects. However, Trudeau et al. were able to detect IgE antibodies to cat flea allergens in 33.3% (16 of 48) of cat flea skin test-positive sera of individuals in the Tampa Bay area of Florida, and the allergens were different from the antigenic components of the salivary cat flea (59). These results suggest a mode of sensitization other than by bite. Furthermore, using their in-house flea extract, flea allergens were quantified in eight house-dust samples using RAST inhibition assays. Increasing evidence such as this indicates that insects are a significant source of both indoor and outdoor inhalant allergens.

A new inhalant allergy is associated with the Asian lady beetle or ladybug (*Harmonia axyridis*). This insect was introduced in 1916 into the United States and released until 1990 as an ecological control for aphids. The first report of allergy to Asian lady beetle was published in 1999 by Yarbrough et al. and describes two cases of allergic rhinoconjunctivitis. *H. axyridis* seeks refuge in homes during fall and winter, leading to patient sensitization and allergic symptoms including rhinitis, wheezing, urticaria, conjunctivitis, chronic cough, and asthma (60,61). Reports on ladybug allergy come from St. Louis (Missouri), Appleton (Wisconsin), Louisville (Kentucky), West Virginia, and Georgia, and a survey reveals positive responses in North Central, Mid-Atlantic, and New England states (60,61). Five ladybug proteins, with molecular weights of approximately 8.6, 21, 28, 31, and 75 kDa, bind IgE antibody in Western blots (60). Partial protein sequences of purified Har a 1 (10 kDa) and Har a 2 (55 kDa), as well as the complete cDNA sequence encoding for two additional ladybug allergens (16.6 and a 30 kDa), have been reported (61,62). Further analysis of the allergens involved in Asian lady beetle allergy will help to better understand this novel inhalant allergy.

COCKROACH ALLERGEN CROSS-REACTIVITY

Inter-Cockroach Species Cross-Reactivity

Allergen cross-reactivity refers to the concordance of skin or RAST reactivity between two or more crude extracts due to the presence in the extracts of homologous proteins from different species that share IgE antibody-binding epitopes. Skin test or in vitro test panels are unlikely to identify primary sources of sensitization without adequate histories and evidence of exposure. In the attempt to control allergic disease by reducing allergen exposure, it is necessary to minimize exposure to all sources of the sensitizing and the cross-reacting allergens. Cross-reactivity studies clarify exposure patterns that are reflected in skin or in vitro test results and define important shared or unique allergens for further study.

Although some of the cloned cockroach allergens from *B. germanica* (i.e., Bla g 5) and *P. americana* (i.e., Per a 3) are species specific, cross-reactivity occurs among American and German cockroach allergens (63–66). Early clinical studies confirm cross-reactivity between both cockroach species (20,63,67,68). Stankus et al. identified two major acidic cockroach allergens from *P. americana* and *B. germanica* that share allergenic activity using physicochemical techniques and immunoprinting studies (65). Allergenic cross-reactivity is proven between the Madagascar hissing cockroach (*Gromphadorhina portentosa*), a popular pet, and other four cockroach species (69). An analysis of 45 antigens in *P. americana* and 29 antigens in *B. germanica* by crossed immunoelectrophoresis and immunoblots identified Per a 1 and Bla g 1 as cross-reactive homologous allergens from *P. americana* and *B. germanica*, respectively (64). Subsequent molecular cloning reveals that the Group 1 cockroach allergens is a mixture of allergenic proteins of different sizes (6, 21, 32, 43 kDa up to 90 kDa) (31,70). A 70% to 72% amino acid identity between Bla g 1 and Per a 1 reveals the molecular basis of allergenic cross-reactivity between both allergens (30–33).

Other allergens are also responsible for the cross-reactivity among different cockroach species. As mentioned, tropomyosins constitute the Group 7 cockroach allergens (52–54). Bla g 7 shares approximately 98% amino acid sequence identity with Per a 7. The dusky brown cockroach (*P. fuliginosa*) also produces a tropomyosin with 100% and 98.2% amino acid identity to Per a 7 and Bla g 7, respectively (71). Since tropomyosin is a common protein in arthropods, cross-reactivity among species in this big taxonomic group has been described (section Extra Species Cross-Reactivity). However, a comparative study of the relative importance of five cockroach allergens reveals that the prevalence of sensitization to tropomyosin among

cockroach-sensitized patients is low (~13% to Per a 7) compared to other cockroach allergens (54% to rBla g 2, 26% to rBla g 1, 17% to rBla g 4, and 37% to rBla g 5) (36).

Other cockroach allergens have been cloned that share homology between German and American cockroach and most likely also contribute to inter-cockroach species cross-reactivity. The Group 6 of cockroach allergens (troponin C), for example, shows a 14% prevalence of IgE antibodies among cockroach-sensitized patients (51). Additionally, an arginine kinase from *P. americana* has been described as a major allergen for cockroach-allergic Thai patients and cloned (accession number AY563004) (72). A German cockroach homolog is included in the GenBank (accession number ABC86902). Continued recognition and identification of cockroach allergens will help to understand and guide the proper management of cockroach-induced atopic disease.

Extra Species Cross-Reactivity

Several reports in the literature prove cross-reactivity between allergens from cockroach and other species. Witteman et al. showed that IgE antibodies in patients' sera react with silverfish, cockroach, and/or chironomid extracts in 30% of house-dust mite-allergic patients in the Netherlands (73). RAST inhibition studies identified a cross-reactive allergen among members of the groups Crustacea, Arachnida (*Dermatophagoides pteronyssinus*), and Insecta (*B. germanica*) (74).

Tropomyosin, a protein involved in muscle contraction, is also a cross-reactive allergen among members of the phyla Arthropoda and Mollusca (75-77). The Arthropoda producing allergenic tropomyosin include species from Crustacea (shrimp, crab, lobster, crawfish), Arachnida (dust mites), and Insecta (cockroaches, chironomids, silverfish). The Mollusca include Bivalvia (oysters, mussels, scallops, clams, pen shells), Gastropoda (snails, abalones, whelks), and Cephalopoda (squids, octopus, and cuttlefish). For example, tropomyosin could be the cross-reactive allergen in IgE-binding components between boiled Atlantic shrimp and German cockroach in the studies performed by Crespo et al. (78). An insect tropomyosin from *Lepisma saccharina*, Lep s 1, is also immunologically characterized and shows cross-reactivity with rPer a 7, the dust mite rDer p 10, and natural shrimp tropomyosin (79). These invertebrate tropomyosins share an approximately 80% amino acid sequence identity, whereas they are only approximately 45% homologous to human and edible meat (chicken, beef, pork, lamb, etc.) tropomyosins. This difference in homology may explain why humans do not develop allergies to edible meat tropomyosin (38). Interesting observations illustrate the clinical relevance of tropomyosin cross-reactivity. Exposure and sensitization to a particular food tropomyosin (dietary source) may lead to reactivity to aeroallergen exposure, and vice versa; increased exposure to aeroallergens (such as mite tropomyosin during immunotherapy) may result in reactivity to cross-reacting seafood tropomyosin (80). Immunoglobulin E antibody reactivity to the major shrimp allergen, tropomyosin, occurs in unexposed Orthodox Jews who observe Kosher dietary laws that prohibit eating shellfish. This reactivity is due to sensitization to tropomyosin from other origin (cockroach, mite) (81).

In investigations of crustacean foods and stinging insects, Koshte et al. found IgE antibodies to cross-reacting carbohydrate determinants (CCD) and other cross-reacting antibodies to homologous proteins in extracts of mussels, oysters, shrimps, crabs, and honeybee and yellow jacket venoms (82). An IgE-reactive determinant has been proposed to be the $\alpha(1,3)$ -fucosylation site of the innermost *N*-acetyl glucosamine residue of *N*-glycoproteins, which are frequent in insects and plants. IgE antibodies against nonmammalian *N*-glycans, $\alpha(1,3)$ -fucose, and $\beta(1,2)$ -xylose can result in extensive cross-reactivity among plants and invertebrates (83). Whether these glycans play a prominent clinical role as dominant IgE epitopes or in the synthesis of allergen-specific IgE in vivo is not yet determined.

Indoor, outdoor, and workplace exposure to large numbers of insect species in different geographic regions make it extremely difficult to determine whether multiple sensitivities are explained by multiple exposures or by insect allergen cross-reactivity. From clinical and immunological observations, allergy to a single arthropod is uncommon, and cross-reactivity can extend to foods and other arthropods. The term "pan allergy," sensitization to one or a few insects with allergenic similarities that may extend to other noninsect member of the phylum Arthropods, defines this phenomenon (84).

Other cockroach allergens show cross-reactivity with arthropod homologs. The above-mentioned arginine kinase shares homology with Pen m 2 from black tiger shrimp (*Penaeus monodon*), Der p 20 from house-dust mite (reported in the WHO/IUIS list of allergens), and

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MECHANISMS RELATED TO COCKROACH ALLERGEN SENSITIZATION

Although little is known about the underlying mechanisms of cockroach sensitization, few studies report effects that may contribute to cockroach allergy. Antony et al. observed that American cockroach extracts (lacking serine and aspartic proteinase activity) induce the release of a vascular permeability factor (VEGF) to bronchial airway epithelial cells causing endothelial barrier abnormalities and increased microvascular permeability (88). The authors proposed a mechanism for increased sensitization to cockroach allergens by suggesting that this barrier breakdown facilitates allergen entry into the bronchial airways causing both sensitization and the allergic response. In contrast, Bhat et al. demonstrated that German cockroach extracts contain a serine protease activity, which has a direct inflammatory effect upon airway epithelial cells (89). Serine protease activity in German cockroach extract has been reported (41). Cockroach allergen extracts stimulate protease-activated receptor-2 (PAR-2), which is upregulated in respiratory epithelium from asthmatic patients (90,91). A serine protease (Per a 10) has been added to the WHO/IUIS list of Allergen Nomenclature, but the allergen needs further characterization (Table 3).

Using cultured human epithelial cells, German cockroach extracts synergistically increase TNF- α -induced transcription from the IL-8 promoter (89). Moreover, the IL-8 expression is dependent on a serine protease activity, sensitive to protease inhibitors, but it is not induced by the endotoxin at levels present in cockroach extracts. Rullo et al. investigated the levels of endotoxin, mite, and cockroach allergens in schools and suggested that endotoxin may be capable of inducing airway inflammation and worsening asthma (92). Thus, environmental control of both allergen and endotoxin levels may modify sensitization and allergic response. At the molecular level, the characterization of B- and T-cell epitopes on cockroach allergens will permit a better understanding of the immunopathogenic mechanisms involved in insect hypersensitivity.

DIAGNOSIS AND IMMUNOTHERAPY

Diagnosis

Skin testing using crude whole-body extracts is the gold standard to diagnose cockroach allergy. RAST, basophil histamine release, and total IgE are poor predictors of subsequent bronchial provocation results. RAST has an approximately 50% false-negative rate. At present, cockroach extracts used for skin testing are not standardized, and those commercially marketed are prepared from whole-body extracts of the three most common species: American, German, and Oriental. Assays, using allergen-specific monoclonal antibodies, showed up to 200-fold differences in Bla g 1 levels in six commercial extracts, ranging from 4.7 to 1085 units/mL (U/mL), whereas only two extracts contained detectable Bla g 2 (248 and 324 U/mL) (26). Serological studies suggest that a cocktail of *B. germanica* allergens, Bla g 1, Bla g 2, Bla g 4, and Bla g 5, would diagnose 95% of U.S. patients with cockroach allergy (93). The use of recombinant allergens that can be produced as pure solutions, using in vitro expression systems, should allow diagnosis of sensitization to specific allergens in the future.

Measurement of cockroach allergen exposure may allow prediction of sensitization. As with other indoor aeroallergens, airborne particles carrying allergens cannot be readily identified or counted. There is no equivalent of a pollen count. Counting numbers of cockroaches and mites may be a reasonable guide to the quantity of allergen; however, the best measurements are obtained using immunochemical assays of major allergens in extracts of dust collected from natural sources. Emergency room studies show that individuals with a

positive RAST to cockroach of >40 U/mL have Bla g 2 levels of >2 U/g in house-dust samples. As mentioned in section Public Health Importance of Cockroaches, current evidence suggests that >2 U/g Bla g 2 or Bla g 1 is the "threshold" allergen level for cockroach allergen sensitization (40,94). The risk levels for asthmatic symptoms are 8 U/g Bla g 1 (6).

Immunotherapy

Allergen immunotherapy is effective for patients with insect sting hypersensitivity. However, at present, cockroach immunotherapy is not considered efficacious (93). In a single study, allergen immunotherapy using cockroach vaccines in sensitive individuals decreased symptom scores and medication requirements. It also increased specific IgG levels and decreased basophil histamine release in response to cockroach antigen (95). The use of standardized cockroach extracts could improve cockroach immunotherapy efficacy. However, the U.S. FDA has reported variability of commercially available cockroach allergen extracts in protein content, electrophoretic banding patterns, relative potency, and Bla g 2 levels (96). Efforts to standardize cockroach extracts have been reported and will be necessary to properly develop effective immunotherapy for cockroach allergy (97,98).

As with any other allergy, cockroach allergy therapy should be based on three approaches: (i) environmental control (avoidance) (section Environmental Control), (ii) pharmacotherapy, and (iii) immunotherapy with the appropriate allergens. The use of recombinant cockroach allergens is envisioned as a way to improve therapy of cockroach hypersensitivity. Benefits include better control of batch-to-batch variability and the assurance of representation of minor allergens in standard amounts. Additionally, immunotherapy with specific hypoallergenic recombinant allergens or peptides lacking IgE-binding epitopes rather than crude allergen vaccine mixtures could prove to be a more effective regimen to avoid anaphylactic reactions. Specific immunotherapy with recombinant cockroach allergens, such as performed with cat and mite allergens, has yet to be performed.

ENVIRONMENTAL CONTROL

Advances in integrated pest management include preventing or minimizing populations within structures. Manipulations of microclimates in discrete areas of new homes can and does reduce infestation. Methods include incorporation of nontoxic repellents in structures to deny access, such as beneath sinks in kitchens and bathrooms. As in any management scheme, environmental control and reduction in allergen levels are the main objectives for asthma-related illness management. Additionally, monitoring allergen levels by individuals in their own homes should improve their understanding of the role of allergens in asthma and improve compliance with future avoidance measures.

For most inhalant allergens, the actual amount of allergen inhaled during natural exposures is low, but the inhaled particles can have very high allergen content. Thus, for environmental control, it is mandatory, not only to remove cockroaches but to remove dust-containing particles carrying the allergen. Control measures should include removal of food and water sources from the natural habitat areas. Increased airflow, maintenance of dryness, and removal of any potential food sources will facilitate environmental control in kitchen cabinets, under sinks and kitchen floors where high concentrations of cockroach allergen are found.

Although these recommendations are sound for some dwellings, heavily infested homes and buildings that contain multiple apartments will be more difficult to control. Re-infestation from neighboring apartments, inadequate eradication and prevention measures, and poor construction and sanitation are sometimes obstacles that may be difficult to control. A significant problem is the determination of allergen levels. Assays currently being used must be standardized to define the relationship between different cockroach species and other cross-reacting allergen sources.

Cockroaches can be controlled by using a variety of chemicals, including organophosphates, carbamates, and botanicals, such as pyrethrins and pyrethroids, which disrupt

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the insect's nervous system, causing locomotion and respiratory failure. Other materials, including wettable powders, emulsified concentrates, aerosols and baits, have been added to the pesticide management of these pests. Ingestion of boric acid leads to damage of the epithelial cells in the gut, precluding nutrient absorption that leads to subsequent starvation. Newer formulations containing active ingredients that interfere with metabolic activity and growth regulation are being used as baits for foraging cockroaches. Although currently available pesticide use (abamectin, hydramethylnon) can reduce populations by 93% to 100%, cockroach allergen found in feces, cast skins, and body parts remain in accumulated dust. Sarpong et al., using several rooms in a college dormitory as a model for home extermination studies, showed that Bla g 2 allergen levels of dust of 5.2 U/g could be reduced to 0.95 U/g following an extermination regimen and regular vacuuming (99). Cockroach control alone can significantly reduce cockroach allergens in infested homes (100). However, sustained decrease of cockroach allergens is difficult to achieve, even after successful extermination of cockroach populations, and the levels may remain above those reported to be clinically significant (reviewed in Ref. 93). Pesticide treatment should be rotated to reduce the risk of resistant strains, and careful cleaning and maintenance are essential to remove and/or reduce the allergen load. Despite limited evidence, reduced exposure to cockroach allergens in infested structures could lead to improvements in asthma morbidity among cockroach-sensitized patients (reviewed in Refs. 101 and 102).

The current recommendations for cockroach control include both physical and chemical measures. Table 5 identifies several cockroach control techniques. Approaches of biological control have been tried or are under development, such as the use of the hymenopter parasite of the ootheca, *Comperia merceti*, against *Supella longipalpa*, or the potential use of a *P. fuliginosa* densovirus as a biopesticide (103). However, these biological approaches to environmental control are still far from being commercially developed. An integrated pest management strategy consisting of sanitation, landscape management, and a perimeter insecticide treatment applied according to label directions is the best control measure possible. Although extensive measures are available to control cockroach populations, neither control procedures for reducing allergen levels nor the extent of cockroach allergen stability and allergen persistence in the environment following cockroach eradication measures are known.

Table 5 Cockroach Control Measures

I. Physical measures
A. Reduce access to food
1. Store food in sealed containers
2. Eliminate sources of organic debris
B. Reduce access to water
1. Repair leaking faucets
2. Wrap pipes to prevent condensation
3. Eliminate damp areas beneath sinks
4. Repair damp, damaged wood
C. Improve ventilation by eliminating clutter beneath sinks
D. Eliminate hiding places and access points
1. Caulk and seal cracks and crevices in foundations
2. Caulk around water pipes entry into house and beneath sinks
3. Eliminate clutter within household (e.g., remove all newspaper and magazine storage areas)
II. Chemical measures
Aerosol sprays of organophosphates like chlorpyrifos were banned (i.e. pesticide diazinon was banned on June 8, 2000; EPA banned indoor use in December 2002)
1. Pyrethrum or pyrethroids
2. Boric acid powders and baits
3. Orange guard (D-limonene)
4. Bait stations
a. Hydramethylnon (Combat)
b. Abamectin (Roach Ender)
c. Fipronil (Maxforce Roach Bait Station)
d. Roach Free™ System (food source with boric acid)
e. Baits with other active ingredients (sulfuramid, xanthines, oxypurinol)

SALIENT POINTS

- Sensitization to indoor inhalant allergens is strongly associated with the development of asthma. In urban and inner-city areas, up to 80% of children with asthma may have IgE antibody to cockroach allergens.
- Infestations of domiciliary cockroaches are largely dependent on housing conditions. The average American spends approximately 95% of the time indoors in controlled environments that lead to continued low-dose allergen exposure, which may lead to sensitization in predisposed individuals.
- Amorphous cockroach particles containing allergens are recognized as an important source of indoor allergens, together with dust mite particles.
- Cross-reactivity of arthropod allergens can be identified among members of the taxonomic groups Crustacea, Arachnida, and Insecta, described as "pan allergy."
- The cloning of several cockroach and other insect allergens has been accomplished using molecular biology techniques. These studies offer the basis for investigating the relationship between allergen function/structure and allergenicity.
- The X-ray crystal structure of Bla g 2 reveals features that contribute to allergenicity. The unusual structure of the area corresponding to the catalytic site explain why this potent allergen does not have standard aspartic protease activity, which is not necessary for allergenicity.
- Recombinant cockroach allergens that retain IgE antibody-binding capacity are new tools that can be used in the future to improve the diagnosis and treatment of cockroach hypersensitivity.
- Environmental control of cockroach and other insect infestations is essential to control inhalant insect-allergic diseases.
- The composition of environmental dust includes a wide range of components from the biosystem and, given the widespread distribution of insects in the world, their involvement in allergic reactions will continue to be of major social, economic, and medical importance.
- Future directions for research should include the study of cockroach reduction strategies, development of specific assays to detect clinically relevant insect inhalant allergens, measures to reduce exposure to environmental allergens, including patient education for pest management and the safe use of insecticides and nontoxic traps, and the study of the mechanisms of cockroach allergy.

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