

Review

Molecular Characteristics of Cockroach Allergens

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Cockroaches, commonly found in urban dwellings worldwide, have long been considered vectors of various infectious diseases and cockroach allergens are one of the major etiologic risk factors for IgE-mediated allergic respiratory illness throughout the world. A high prevalence of cockroach hypersensitivity in atopic (20-55%) and asthmatic (49-60%) populations has been documented. Cockroach allergens with molecular weights ranging from 6 to 120 kD have been identified by various standard immunochemical techniques. This article covers the characteristics of major cockroach allergens that have been purified, sequenced, cloned, and produced as recombinant proteins. *Cellular & Molecular Immunology*. 2005;2(3):177-180.

Key Words: hypersensitivity, asthma, cockroach, cockroach allergen, cloning, recombinant protein

Introduction

Cockroaches, commonly found in urban dwellings worldwide, have been considered vectors of various infectious diseases (1). Among 3,500 known species of cockroaches only 5, the American cockroach (*Periplaneta americana*), German (*Blattella germanica*), Oriental (*Blatta orientalis*), smokey brown (*Periplaneta fuliginosa*), and brown-banded (*Supella longipalpis*) varieties, are frequently found in homes and have the potential to contribute to indoor allergens (2).

Sensitization

Aerosolized proteins derived from saliva, fecal material, secretions, cast skins, debris and dead bodies of cockroaches induce IgE-mediated hypersensitivity. Among airborne allergens, those from the cockroach play a very important role in allergic diseases, especially asthma (3-7). Bernton and Brown (8) were the first to document positive skin test responses to cockroach allergen in 1964. In 1979, Kang et al. (9) established the causal relationship between cockroach allergy and asthma after inhalation of cockroach by sensitized asthmatic patients. Subsequently, the high prevalence of 40%

to 60% of patients with asthma have IgE to cockroach allergens have been reported in several urban or inner cities around the world (10) including Taiwan (11). Several studies (10, 12, 13) have indicated that most patients sensitized to cockroaches were exposed to high levels of cockroach allergens in their homes and that cockroach allergy is an important risk factor for emergency department visits for asthma and hospital admissions. Recently, the national Cooperative Inner City Asthma study (14) has confirmed that the association of sensitization and exposure to cockroach allergens is a major risk factor for morbidity caused by asthma in children from large cities in the United States. A more recently prospective study (15) has demonstrated a significant association between exposure to cockroach allergens in the first 3 months of life and the development of repeated wheeze in the first year among children in metropolitan Boston.

Allergens

Allergens with masses ranging from 6 to 120 kD from both *P. americana* and *B. germanica*, the most common domiciliary cockroach species, have been identified by various immunochemical techniques (3, 10). Previously, we identified two partially purified allergenic fractions, Cr-PI [Per a 3] and Cr-P11 [Per a 1], from crude American cockroach extract (16). Two prominent proteins of 78 and 72 kD in Per a 3 cause T cell proliferation in cockroach allergic patients (17), and three proteins of 45, 32 and 28 kD in Per a 1 (18) have been identified as major and principal *P. americana* allergens. Over the last ten years, three *P. americana* allergens, Per a 3 (19-21), Per a 1 (22-24) and Per a 7 (25, 26), and six *B. germanica* allergens, Bla g 1 (27), Bla g 2 (28), Bla g 4 (29), Bla g 5 (30), Bla g Bd90K (31) and Bla g 6 (10), have been cloned.

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Table 1. Immunochemical properties of cockroach allergens

Species	Allergen	Prevalence of IgE antibody (%)	Molecular weight	Function
American cockroach	Per a 1	54-77	26-45 kD	Unknown
	Per a 3	26-95	56-79 kD	Insect hemolymph
	Per a 7	50	33 kD	Tropomyosin
German cockroach	Bla g 1	30-50	20-15 kD	Unknown
	Bla g Bd90K	77	90 kD	Unknown
	Bla g 2	60	36 kD	Aspartic protease
	Bla g 4	40-60	18 kD	Calycin
	Bla g 5	70	23 kD	GST
	Bla g 6	50	18 kD	Troponin C

Molecular characteristics of allergens

Molecular cloning techniques have been used to sequence a few cockroach allergens and to investigate their biochemical activities and normal biological roles. American and German cockroach cDNA expression libraries have been screened with human atopic IgE or murine monoclonal antibodies to identify positive clone expression of the allergen. This approach allows the rapid determination of allergen primary structure and production of recombinant allergen proteins for detailed characterization of linear B- and T-cell epitopes. Most cockroach allergens appear to be species-specific. The only cross-reactive allergens that have so far been sequenced from both *P. americana* and *B. germanica* are the group 1 allergens Bla g 1 and Per a 1 (22-24, 27, 31).

Per a 3 containing isoallergen is a species-specific allergen of the American cockroach that causes IgE antibody responses in 26.3% to 94.7% of patients allergic to cockroaches, suggesting a high degree of polymorphism among the allergens and the potential usefulness of the isovariants in elucidating specific allergenic determinants (21). While there was no sequence similarity with other known proteins, these aromatic amino acid-rich (16.5%-17.3%), Per a 3 allergens were found to have striking sequence identities (20.1%-36.4%) to insect hemolymph proteins (19-21). Other circulatory fluids, including hemolymph and hemoglobins, may contribute to the repertoire of insect allergens. The similarity of a lipopolysaccharide-binding protein from the hemolymph of the American cockroach with other insect hemolymph proteins and with animal lectins also suggests that this class of protein may be allergenic (32). Chi t 1, the hemoglobin from the European midge species, *Chironomus thummi*, represents the major allergenic component causing rhinitis, conjunctivitis and asthma in exposed populations. Immunological cross-reactivity has been found between hemoglobins of closely related Chironomidae species (33). These results suggest the hemoglobins and hemocyanins of insect may also represent an important source of arthropod allergens. Per a 1 molecule containing isoallergen shares high sequence homology (51.6%-70.9%) with Bla g 1 including originally reported as Bla g Bd90K of German cockroach and both molecules

contain internal repeated sequence, phosphorylation sites, mitochondrial energy transfer protein signatures, and contain no cysteine and potential N-glycosylation site (22-24). Per a 1 showed 54.4% to 77.3% skin reactivity in atopic patients, and was also found to have significant 27.0% to 32.0% sequence identity to the precursor protein of the female African mosquito (*Anopheles gambiae*) that is induced following a blood meal. Per a 7 have been recently identified. It shows high degree of sequence identity to tropomyosins from invertebrates, particularly from mites (80%), shrimp (82%), and snails (25, 26). Tropomyosin has been previously reported as important allergens mite (Der p 10 and Der f 10) and shrimp (34-36). It is possible that tropomyosin may play the role of cross-reactivity among mites, cockroaches, and the shrimp and that the high degree of sequence identity has clinical significance (37).

Bla g 2 shows homology to aspartic protease, including pepsin, cathepsin, and chymosin (28). The prevalence of IgE to Bla g 2 among cockroach atopic patients ranges from 60% to 80%. Bla g 4 is a member of ligand binding proteins (also known as calycins or lipocalins), and the prevalence of IgE to Recombinant Bla g 4 is 60% (28). Calycins represent a family of proteins that include several other important allergens, including β lactoglobulin from cows' milk and rat, mouse urinary proteins. Bla 5, showing 40% prevalence to IgE in atopic patients, is a member of the glutathione-S-transferase (GST) family of enzyme (29). It shows 50% homology to other insect GSTs and 28% homology to house dust mite GST allergen Der p 8. GSTs are enzymes involved in the detoxification of endogenous and xenobiotic toxic compounds, and their production in insects is associated with resistance to insecticides. Therefore, it is possible that GST allergen production could be upregulated by the use of insecticides. The cDNA for Bla g 6 encodes a protein of an estimated mass of 21kd, which shows homology to troponin-C (10). A list of the cockroach allergens identified is shown in Table 1.

Epitopes of the allergens

Although several cockroach allergens have been cloned and sequenced, information concerning epitopes of cockroach

allergens has been unavailable so far. We reported previously that IgE-binding epitopes of Per a 1 isoallergen, clone C42, were located in the internal repeats of molecule (23). Recently, by the same approach, deletion mutants were generated from Per a 1 clone C17 allergen (274 amino acid residues) (19). The deletion mutants 1-77, 86-205, and 200-266 of clone C17 were found unable to recognize atopic human IgE. On the other hand, an N-terminal fragment 1-87, and C-terminal deletion mutant 200-274 were recognized by atopic human IgE, and had comparable reactivity to IgE as full-length Per a 1. The amino acid sequences 78-85 and 267-274 were revealed as the minimum amino acid sequences required for IgE binding of Per a 1 allergen that are in the internal repeated sequences of Per a 1 (38). More recently, specific and predetermined deletion mutants were generated from Per a 3 clone C12 allergen (685 amino acid residues) (20) by existing restriction sites or using polymerase chain reaction products. The cDNA was expressed as recombinant proteins in *Escherichia coli*, and the reactivities of these partial Per a 3 molecules to IgE were examined by immunoblotting in our laboratory. The N-terminal fragment 1-399, deletion mutants 410-443, 472-551, 502-579, 606-636 and C-terminal fragment 636-685 were unable to recognize atopic human IgE. On the other hand, atopic human IgE recognized deletion mutants 340-425, 378-474, 466-579, 502-595 and 595-636, and deletion mutants 466-579 and 595-636 had comparable reactivity to human IgE as full-length Per a 3. The amino acid sequences 400-409, 466-471, 580-595 and 595-605 were revealed as the minimum amino acid sequences required for IgE binding of Per a 3 clone C12 allergen, suggesting that the C-terminus of the Per a 3 contain most of the IgE-binding sites (39).

Conclusions

Sensitization to indoor inhalant allergens is strongly associated with the development of asthma. In urban and inner-city areas, 40% to 60% of patients with asthma have IgE antibody to cockroach allergens. Amorphous cockroach particles containing allergens are recognized as significant indoor allergens second only to that of the dust mites. Recombinant cockroach allergens could potentially be used to standardize crude extracts or used in a modified form for immunotherapy. The use of recombinant cockroach allergens that retain IgE recognizable epitopes has been envisioned to provide the basis for improving therapy for persons suffering cockroach hypersensitivity. Immunotherapy with specific recombinant allergens or epitopes rather than crude allergen vaccine mixtures could prove to be a more effective regimen. Benefits include better control of batch-to-batch variability and the assurance of representation of minor allergens in standard amounts.

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