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Every Cockroach Is Beautiful to Its Mother

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Cockroaches are considered to be among the oldest insects on our planet, predating dinosaurs by more than 150 million years and humans by more than 300 million [1]. Some fossils attributed to cockroaches date back to the Carboniferous period, around 325 million BC. However, an uncertain evolutionary link between cockroach-like fossils from the Carboniferous period (Paleozoic era) and 'modern' cockroaches, which originated in the Cretaceous period (Mesozoic era) as did most other insects, has also been suggested, due to morphological differences [2]. Regardless of their origin, cockroaches have undergone very few changes over time, and the diversification of species and adaptation to multiple habitats are remarkable, reflecting their tremendous success at surviving throughout evolution [1]. Over 4,000 cockroach species have been identified, with tropical forests as their preferred habitat [3]. However, a few species, including the German cockroach *(Blattella germanica)* and the American cockroach *(Periplaneta americana)*, brought from Africa to the Americas on slave ships, have adapted to live near people, turning into household pests.

The Neapolitan folk saying *Ogni scarrafone è bello a mamma sua* (Every cockroach is beautiful to its mother) may soften the disgust towards the cockroach. Among the general population, there is strong concern that cockroaches can serve as vectors of diseases, representing a threat to human health. Although cockroaches may harbor bacteria, viruses, fungi and parasites, dissemination of diseases by cockroaches is not easy to document, given the precarious hygienic conditions of some of the habitats where they live. In fact, asthma is the only disease consistently associated with exposure to cockroaches [4–6]. Curiously enough, cockroaches have no lungs (they breathe through lateral spiracles in the body) [1], but inhalation of cockroach materials by humans may trigger IgE antibody responses, lung eosinophilic inflammation and asthma attacks. The first reports on exposure to cockroach and IgE sensitization were published in the early 1960s [7, 8]. Subsequent studies have established an important role of exposure and sensitization to cockroach in causing earlier onset and more severe symptoms of asthma, particularly among inner-city and underprivileged populations, at lower doses of allergen as compared to other allergen sources [9].

How do people become sensitized to cockroaches? Where do cockroach allergens come from? What is special about cockroach allergens? Those are very important questions. In the past 20 years, advances in understanding cockroach allergy have been possible through the identification, characterization, molecular cloning and recombinant expression of cockroach allergens [4, 5]. In addition, development of assays for measuring cockroach allergens (Bla g 1 and Bla g 2 in particular) has been fundamental to establish the importance of environmental exposure to cockroach in asthma [10].

Cockroach debris, secretions and cast-overs are thought to be important sources of allergens [6]. Allergen

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exposure may be anticipated when the cockroaches die or molt, or when their dried secretions from the digestive or reproductive systems become part of the indoor dust. Structural biology studies demonstrate that the allergens Bla g 1, Bla g 2 and Bla g 4 are proteins secreted or excreted by cockroaches. On the other hand, allergens with structural functions, like the group 7 tropomyosins or allergens from groups 6 and 8, are likely to be released after degradation of dead bodies [5]. These dried secretions and remains of body parts would constitute the form in which cockroach allergens become airborne, carried mostly by particles larger than 10 μm that are detectable mainly after vigorous activity, settle rapidly and induce sensitization in genetically predisposed individuals [6]. It has been hypothesized that certain properties of cockroach allergens, including proteolytic activity, could increase their allergenic potential and therefore contribute to the severity of disease associated with cockroach exposure and sensitization [11]. However, most cockroach allergens identified to date by molecular cloning are non-proteolytic, with the exception only of Per a 10, which has serine protease activity [11, 12].

Interestingly, there is no dominant cockroach allergen, as has been described for other allergen sources, including cat (Fel d 1), mites (group 1 and group 2 allergens) or pollens (Bet v 1, Amb a 1). Profiles of IgE reactivity to cockroach allergens are variable when comparing individual cockroach-allergic patients and among different populations [13]. Several proteins belonging to 10 distinct groups, with diverse functions in the cockroach, have been identified as allergens and are reported in the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database (www.allergen.org). *B. germanica* produces the following allergens: Bla g 1, a secreted midgut microvilli protein homolog; Bla g 2, an inactive aspartic proteinase; Bla g 4, a lipocalin, secreted only by the male cockroach during reproductive activity; Bla g 5, a glutathione S-transferase, suggested to be important for detoxication of endogenous and xenobiotic toxic compounds, and proteins involved in muscular contraction, i.e. Bla g 6 (troponin), Bla g 7 (tropomyopsin) and Bla g 8 (myosin light chain). *P. americana* expresses the following allergens: Per a 1, analog to Bla g 1; Per a 3, homologous to arylphorins and insect hemocyanins; Per a 6 and Per a 7, analogs to Bla g 6 and Bla g 7; Per a 9, an arginine kinase involved in the metabolism of ATP, and Per a 10, a serine protease. In addition to the list in the WHO/IUIS database, other allergen homologs from cockroach have been described, i.e. *P. americana* proteins which share homology with *B. germanica* group 2 and 4 allergens and with mite allergens from groups 2, 3 (trypsin) and 13 (fatty acid binding protein). These proteins are not yet included in the official allergen database.

A study by Satinover et al. [13] made it evident that the most important cockroach allergens at that time, identified by screening cDNA expression libraries and reported in the WHO/IUIS Allergen Nomenclature database, did not cover the entire allergen repertoire of the German cockroach. The prevalence of IgE antibodies among cockroach-allergic patients living in the USA was highest for rBla g 2 (50–72%) and rBla g 5 (35–60%). Thirty-six percent of 114 allergic patients reactive to cockroach extracts did not recognize any of the allergens from groups 1, 2, 4, 5 and 7, and, therefore, these 5 allergens did not account for the total IgE reactivity to cockroach. Proteomics arose as an alternative technology for the identification of additional allergens that were still to be discovered in cockroach.

In this issue of *International Archives of Allergy and Immunology,* Jeong et al. [14], from Korea, have used a proteomics approach to identify novel cockroach allergens in a *B. germanica* fecal extract. As could be predicted, they have found that 4 of 12 IgE-binding proteins were digestive enzymes, including midgut carboxypeptidase A, chymotrypsin, astacin-like metalloprotease and trypsin. α-Amylase was found to be an important allergen with a 41% prevalence of IgE reactivity. A previous proteomics study published in 2010 by Chuang et al. [15] in Taiwan used a whole-body extract of the German cockroach, instead of fecal extract, to identify 10 new candidate allergens. They reported aldolase, enolase, heat shock protein Hsp70, triosephosphate isomerase and vitellogenin as new cockroach allergens. Vitellogenin had the highest prevalence of IgE reactivity (48%) after Bla g 2 (63%). Differences in the proteins identified by both proteomic studies may be due, in part, to the source of the cockroach material used for immunoblotting. Accordingly, Chuang et al. [15] did not identify Bla g 1 or Bla g 2, in agreement with expected very low levels in whole-body extracts from starved cockroaches compared to fecal extracts. In addition, female insects at the oviposition stage were used, which would have very low levels of Bla g 1.

Jeong et al. [14] have focused their study on isoforms of α-amylase sequenced from IgE-binding spots identified using two-dimensional (2D) electrophoresis and IgE immunoblotting analysis. Amylase activity was previously reported from various cockroach gut compartments and salivary glands. German cockroach α-amylase shares the highest identity with pig α-amylase (55.8%) and group 4 mite allergens (Blo t 4, 50.4%; Der p 4, 49.8%; Eur m 4,

47.4%). These results raise the interesting possibility that α-amylase could be another invertebrate cross-reactive allergen in the cockroach, in addition to tropomyosin, glutathione S-transferase, arginine kinase and myosin light chain, which share homologs in mites, shrimp and parasites [16, 17].

Recombinant cockroach α-amylase expressed in *Escherichia coli* was shown to bind IgE of 41.4% of cockroach-allergic patients [14]. In addition, it inhibited 55% of IgE binding to cockroach whole-body extract. However, IgE binding to this protein appeared to be low on 2D immunoblotting and ELISA. The addition of other methods including skin testing or basophil activation assays could provide further evidence that α-amylase would be an allergen worth pursuing for diagnostic and therapeutic purposes. Also, further studies including a larger number of patients and individuals from different populations would be important to consolidate the role of α-amylase as an allergen.

The success of a proteomic approach in identifying allergens additional to the presently known 10 groups could be partly related to the use of natural allergens in this technology. Most of the cockroach allergens reported in the WHO/IUIS Allergen Nomenclature database were identified by screening expression libraries. This approach required isolation of easily degradable RNA and expression of recombinant proteins by a prokaryotic system (not always ideal for expression of eukaryotic proteins). In contrast, proteomic approaches are based on recognition of natural allergens that preserve some of their native IgE antibody-binding epitopes (even if they are linear, as claimed by the authors for α-amylase). In addition, the populations of cockroach-allergic patients used in the proteomic studies were from Korea and Taiwan, and their genetic background is expected to be different from the American populations previously selected to identify allergens. There may also be differences in the environmental exposure in different countries that are worth investigating. A recent study showed that sensitization to tropomyosin in cockroach-allergic patients with asthma and/or rhinitis living in Brazil is much more prevalent than to the allergens Bla g 1 and Bla g 2, considered major allergens in the USA [18]. Therefore, follow-up studies to evaluate the relative importance of the newly identified allergens in different populations and geographic locations will provide valuable information for our understanding and management of cockroach allergy.

One feature that may make cockroaches so fit for survival is their omnivorous habit, evidenced by their ability to eat practically anything in their environment, even the most repulsive substances [1]. Interestingly, in the current study by Jeong at al. [14], the authors identified a soybean allergen homolog, glycinin, on 2D analysis, leading them to propose that it was derived from the mouse food used to feed the cockroaches in colonies and excreted in feces. The authors raise an issue when considering manufacturing of cockroach extracts, of whether excreted fecal proteins derived from cockroach food could act as contaminants, leading to false-positive results on diagnostic tests.

The important issue now is to determine how we could translate the accumulated knowledge about cockroach allergens into clinical practice, to improve diagnosis and management of our cockroach-allergic patients. Cockroach extracts currently manufactured in the USA for allergy diagnosis present variation in levels of Bla g 1 and Bla g 2 of up to 7-fold [19]. Immunotherapy is also conducted using nonstandardized cockroach extracts. In spite of the relevance of cockroach allergy, there are very few studies on cockroach allergen immunotherapy [20–22]. Although these studies suggest that cockroach immunotherapy may be effective, further randomized, controlled clinical trials would be necessary to determine the efficacy and safety of this form of treatment.

With the knowledge acquired, a panel of cockroach allergens could be designed for testing a large number of patients from different areas of the world, with variable degrees of disease severity, through collaborations among various scientific groups worldwide. In vitro tests including ELISA, streptavidin ImmunoCAP, ImmunoCAP-ISAC, chimeric ELISA and multiplex array assays could potentially improve the diagnosis of cockroach allergy. Analyzing responses to individual allergens could lead to estimates of increased risk for severe disease and predictions of response to immunotherapy. The efficacy of recombinant allergens could be investigated in clinical trials for immunotherapy of cockroach-allergic patients, particularly those at a higher risk for more severe disease such as children and young adults living in inner-city and underprivileged environments.

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