World Allergy Congress 2014

Postgraduate Course 14: Immunotherapy Track – Cockroach Immunotherapy: New Insights

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Cockroach Allergens: Overview of Structure and Function

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Learning Objectives:

- To be able to compare structural and functional features of the most important cockroach allergens, and their contribution to allergenicity.
- To understand how the analysis of antigenic determinants of cockroach allergens can be applied to the design of hypoallergens for immunotherapy.

This year marks the 50th anniversary of the study by Bernton and Brown, published in 1964, that first reported skin test responses to cockroach allergen in patients living in New York [1]. Subsequent work by Kang et al. confirmed the causal relationship between cockroach allergy and asthma by showing early, late-phase, and dual bronchoconstriction after inhalation of cockroach extract by sensitized asthmatic patients [2]. The identification of cockroach allergens and the development of immunoassays to assess exposure has allowed the role of cockroach allergens in causing allergic respiratory disease to be investigated. The National Cooperative Inner City Asthma Study (NCICAS) provided key information on the role of cockroach allergy in asthma. Seminal studies by Rosenstreich and colleagues showed that morbidity among inner-city children with asthma, as indicated by hospitalizations, medical visits and symptoms, was higher among children who were both sensitized to cockroach and

exposed to high levels of cockroach allergen in their homes [3]. The association between cockroach exposure and increased asthma morbidity in inner-city areas in the United States and in other countries has subsequently been confirmed.

German cockroach (*Blattella germanica*) is the most common species in temperate areas like the U.S., whereas the American cockroach (*P. americana*) lives in tropical areas from South American and Asian countries. Ten groups of cockroach allergens are currently listed the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database (www.allergen.org). Cockroach allergens comprise proteins belonging to different structural/functional groups, including digestive proteins (group 1), inactive aspartic proteases (group 2), hemocyanins (group 3), lipocalins (group 4), glutathione S-transferases (group 5), troponins C (group 6), tropomyosins (group 7), myosin light chains (group 8), arginine kinases (group 9) and serine proteases (group 10). Unlike allergens from other sources (i.e. mite and cat), no immunodominant allergens have been described for cockroach [4]. The two most important allergens in the U.S. in terms of prevalence of IgE reactivity are Bla g 1 and Bla g 2 (up to ~70%), whereas Per a 7 was found to be more important in Brazil and Zimbabwe [5,6]. In these countries, sensitization to cockroach allergens could be influenced by co-exposures to tropomyosins from other sources such as Ascaris [6].

Recent analyses of the three-dimensional structure of cockroach allergens have revealed molecular features associate with allergic disease. The X-ray crystal structure of Bla g 1 was reported earlier this year: a challenging task due to the unusual fragmentation pattern of this allergen [7]. Bla g 1 comprises multiple consecutive amino acid repeats resulting from gene duplication of a ~100 amino acid domain [8]. The novel structure is formed by a series of

capsule-like structural units that repeat like beads on a string and contain lipids in their cavity. Bla g 1 could have a digestive function by being involved in the uptake of dietary lipids. Allergens that bind hydrophobic ligands are potent stimulators of the innate immune system and could be involved in skewing towards Th2 responses. The Bla g 1 structure also provided a rationale to understand why the molecule preserves antibody epitopes, despite its variable fragmentation pattern. Bla g 1 levels have been reported in relative units in all environmental studies. Bla g 1 levels vary by up to 200-fold in cockroach extracts used for immunotherapy. One units of Bla g 1 was equal to 104 ng protein, thus providing absolute quantification of Bla g 1 in allergenic products and in environmental exposure assessments [7].

Bla g 2 is an inactive aspartic protease, despite of having a fold typical of this group of proteolytic enzymes [9]. Detailed analysis of the antigenic determinants of Bla g 2 have been performed by determining the X-ray crystal structures of allergens in complex with fragments of monoclonal antibodies (mAb) that inhibit IgE antibody binding [10,11]. The involvement of carbohydrates on the interaction of an antibody with the allergen was analyzed at the atomic level [11]. These molecular structures, combined with detailed site-directed mutagenesis studies, revealed IgE antibody binding sites on Bla g 2 [12]. Structural analyses of cockroach allergens will provide the basis for the design of hypoallergens for immunotherapy.

References

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