STRUCTURAL AND ANTIGENIC STUDIES OF COCKROACH ALLERGENS AND THEIR RELEVANCE TO ASTHMA

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1. INTRODUCTION

Allergic reactions to cockroach (CR) were first described over 30 years ago by Bernton and Brown, amongst patients presenting to their allergy clinic in New York (1). These observations were followed up by several groups, notably by Dr. Kang and colleagues in Chicago in the 1970's, and it is now well established that CR cause IgE antibody responses; that these responses are often associated with asthma; and that the prevalence of CR asthma in some populations in the U.S. is comparable to that of other common aeroallergens, such as dust mite, cat, and pollens (2, 3). Atopic individuals who live in CR infested housing become sensitized by inhalation of potent CR allergens and produce vigorous IgE antibody (ab) responses. There is a strong association between the development of IgE ab to CR and asthma. In some towns and cities in the U.S., up to 60% of house dust allergic, asthmatic patients become sensitized to CR (2–6). Allergic reactions to CR are not confined to urban or inner city populations, but occur wherever housing conditions sustain extensive cockroach infestation. For example, in Louisville, Kentucky, no differences were found between the prevalence of CR allergy in patients living in inner city or rural areas, with ~40% prevalence of positive skin test reactivity in both groups being reported in a 10 year retrospective study (7). IgE ab to CR is an important risk factor for Emergency Room admission with asthma and is most commonly found among lower socio economic groups living in sub-standard housing, which in the U.S.

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often comprise a high proportion of minority groups (African Americans and Hispanics) (8–9).

“Cockroach asthma” has been reported in several other parts of the world, including South East Asia, Europe, and Central America (reviewed in Ref. 3). In the U.S., the principal domicilliary CR species are *Blattella germanica* and *Periplaneta americana*, whereas in Taiwan and Japan, *P. americana* and *P. fuliginosa* appear to predominate. Until recently, the molecular nature of allergens produced by either CR species had not been defined. Immunochemical studies had identified a series of protein allergens, MW 10–70kd, but there was no sequence data on these allergens and it was difficult to establish the relationships between allergens identified by different research groups (10–15). Two allergens, Bla g 1 and Bla g 2, had been purified using monoclonal antibodies and protein purification techniques, and immunoassays for these allergens have been widely used for monitoring environmental CR allergen exposure (16, 17). Over the past few years, several groups have applied molecular cloning techniques to identify and sequence allergens from either *B. germanica* or *P. americana*. The aims of these studies are to establish the allergen structures, physical properties and biologic function; to develop expression systems for producing recombinant allergens; and to use the sequence information to study the immune response to CR allergens in patients with asthma.

2. MOLECULAR BIOLOGY

2.1. *Blattella germanica*

Two allergens were initially purified from *B. germanica*: Bla g 1, a 20–25kd, highly acidic protein, also produced by *P. americana*; and Bla g 2, a 36kd protein, found primarily in *Blattella* spp. (16, 17). The prevalence of IgE ab to Bla g 1 and Bla g 2, assessed by skin testing or serum IgE ab assay was 30–50-% and 60–80%, respectively, and some patients were strongly sensitive to one allergen, but not to the other. More importantly, ~20% of CR allergic patients did not have IgE ab to either protein, suggesting that CR produce other important allergens. To investigate this possibility, a unidirectional cDNA library was prepared from *B. germanica* mRNA and screened by plaque immunoassay with IgE ab in a CR allergic serum pool, with a large panel of individual sera from CR allergic patients, and with hyperimmune mouse polyclonal IgG ab to Bla g 2.

Screening the *B. germanica* cDNA library identified six clones which reacted with IgE ab. We initially focused on a clone which reacted strongly with IgE antibody in 46–64% of sera and sequence analysis showed that the cDNA encoded a ligand binding protein or calycin (18). This allergen was designated Bla g 4 in the WHO/IUIS nomenclature. Sequence homology searches showed that the calycin protein family also included other major allergens, including mouse and rat urinary proteins, β-lactoglobulin from cows milk, and dog allergens. Since X-ray crystal coordinates were available for α₂u-globulin and bilin binding protein (a butterfly calycin), three dimensional models of the tertiary structure of Bla g 4 were obtained, which predicted two possible structures differing at a loop region between the α-helix and C-terminal β strand (18).

Five other CR allergen clones were obtained from the *B. germanica* cDNA library by IgE ab screening and two clones were fully sequenced. Bla g 5 showed sequence homology to Glutathione-S-transferase enzymes (GST’s) and elicited IgE antibody responses in ~70% of CR allergic patients (19). Bla g 6 showed up to 70% homology with troponins and gave positive IgE antibody plaques in 50% of patients. The sequence homology be-