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Molecular Cloning of German Cockroach (*Blattella germanica*) Allergens

Key Words

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Abstract

Allergens produced by cockroaches (CRs) are an important cause of IgE antibody responses and asthma. Using molecular cloning and nucleic acid hybridization techniques, we have identified and sequenced several important allergens produced by the German CR (*Blattella germanica*) and studied their expression in the American CR (*Periplaneta americana*). Principal allergens include Bla g 2 (36-kD protein) and Bla g 4 (21-kD protein), to which 60–70% of CR-allergic patients make IgE antibodies. Bla g 2 is only expressed by *B. germanica*, whereas DNA encoding Bla g 4 is present in *P. americana*, but is not transcribed into mRNA. Sequence homology searches have identified Bla g 2 as an aspartic protease and Bla g 4 as a calycin. Other CR allergens that have been cloned include a glutathione transferase and a troponin. These studies will enable recombinant allergens to be expressed and used to investigate the role of CR allergens in asthma.

Introduction

Atopic individuals who live in cockroach (CR)-infested housing become sensitized by inhalation to potent CR allergens and produce vigorous IgE antibody (Ab) responses. There is a strong association between the development of IgE Ab to CRs and asthma. In some towns and cities in the US, up to 60% of house-dust-allergic, asthmatic patients become sensitized to CRs, and IgE Ab to CRs is an important risk factor for emergency room admission with asthma [1–4]. 'Cockroach asthma' has been reported in Taiwan and SE Asia [5]. Prolonged exposure to CR allergens in the workplace also causes occupational asthma. In the US, most CR-allergic patients are from lower socioeconomic groups, containing a disproportionate number of African-Americans,

and these patients are often uninsured [3, 4]. The principal household CR species are *Blattella germanica* and *Periplaneta americana*. Most asthma in the US is associated with sensitization to *B. germanica*, whereas in Asia, *P. americana* appears to predominate.

The molecular nature of allergens produced by both of these CR species has not been defined. Two allergens, Bla g 1 and Bla g 2, have been purified using monoclonal Abs (mAbs) and protein purification techniques, and immunoassays for these allergens have been widely used for monitoring environmental CR allergen exposure [6, 7]. We have used molecular cloning techniques to sequence CR allergens, to investigate their expression in different CR species and to analyze their protein structure.

Materials and Methods

A unidirectional cDNA library was prepared from *B. germanica* mRNA in the UniZAP-XR vector (Stratagene) and screened with IgE Ab in serum pooled from eight CR-allergic patients with asthma. Six positive plaques from the *B. germanica* cDNA library were cloned and screened against 20–73 individual sera from CR-allergic patients by plaque immunoassay. The library was also screened using hyperimmune mouse polyclonal IgG Ab to Bla g 2. cDNA clones were sequenced by dideoxy chain termination. Sequence similarity searches were carried out using the FASTA program to screen protein data bases. Molecular-modelling studies were carried out using CONGEN and SYBYL to determine homology with X-ray crystal structures in the Brookhaven Protein Database.

Results

Table 1 summarizes the current data on CR allergens that have been cloned or defined using protein purification techniques. Screening the *B. germanica* cDNA library identified six clones which reacted with IgE Ab. Bla g 4 reacted strongly with 47/73 (64%) of sera tested and sequence analysis showed it to be a lipocalin or calycin [Arruda et al., submitted]. Strikingly, the calycins also include several other major allergens: mouse and rat urinary β -lactoglobulin and a dog allergen. Since X-ray crystal coordinates were available for α_2 -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. A full-length cDNA encoding Bla g 2 was obtained by screening the library with polyclonal mouse IgG Ab. This cDNA encoded a 36-kD protein, which showed sequence homology to aspartic proteases. Partial amino acid sequencing of Bla g 2 protein showed >90% concordance with the deduced cDNA sequence. Expression of Bla g 2 and Bla g 4 in *B. germanica* and *P. americana* was compared by Northern blotting or PCR-based techniques. Northern analysis showed a 1.3-kb mRNA for Bla g 2 which was only present in *B. germanica*. Further, a 1-kb PCR fragment could be amplified from genomic DNA from *B. germanica*, but not from *P. americana*. These results strongly suggest that Bla g 2 is only expressed in *B. germanica*. The 0.65-kb mRNA for Bla g 4 was also only detected in *B. germanica*. However, PCR analysis followed by Southern hybridization revealed that DNA encoding Bla g 4 was present in both CR species. These results suggest that the DNA encoding Bla g 4 is not transcribed into mRNA in *P. americana*.

Five other CR allergen clones were obtained from the *B. germanica* cDNA library by IgE Ab screening and two

Table 1. Immunochemical properties of CR allergens

Source	Allergen	MW kD	Function	Sequence
<i>Blattella germanica</i>	Bla g 1	20–25	unknown	none
	Bla g 2	36	aspartic protease	cDNA
	Bla g 4	21	calycin	cDNA
	Bla g 5	25	glutathione transferase	cDNA
	Bla g 6	~25	troponin	cDNA
<i>Periplaneta americana</i>	Per a 1	20–25	unknown	none
	Per a 3	72–78	unknown	none

Allergens were identified and purified on the basis of IgE reactivity on skin testing, radioallergosorbent test, immunoblots and plaque immunoassay. mAb-based ELISA tests have been developed for Bla g 1, Bla g 2 and Per a 3. Allergens are provisionally designated according to the WHO/IUIS nomenclature.

clones were fully sequenced: Bla g 5, which shows approximately 50% sequence homology to *Drosophila* glutathione transferase, and Bla g 6 which is a troponin. In parallel studies, Wu et al. [pers. commun.] in Taiwan have cloned a 72-kD protein allergen, provisionally designated Per a 3, from a *P. americana* cDNA library.

Discussion

The CR allergen sequences provide the basis for detailed studies of the immune response to these allergens, including analysis of T cell responses and epitope mapping. Bla g 2 is the most well-defined allergen, since its IgE-binding activity has been confirmed by quantitative skin testing and IgE Ab assays on a large panel of CR-allergic sera. mAbs to Bla g 2 have been produced, and both protein and nucleotide sequence data have been determined. For allergens that have been identified by screening cDNA libraries, confirmation of their allergenic activity will require production of the recombinant allergens for skin testing and mAb production, and isolation of the natural allergen for comparative studies.

Sequence homology searches have provided valuable insights into the potential biologic function of the cloned allergens. The rodent calycin allergens are pheromone-binding proteins and we have speculated that the CR calycin, Bla g 4, could also bind pheromones. CRs secrete several different pheromones and there is good evidence that CR

allergens are also secreted/excreted proteins. Similarly, CRs are known to produce high levels of glutathione transferase (for detoxification purposes) and may excrete this protein with feces (another documented source of CR allergens). Comparative studies on the expression of allergens (DNA and protein) in different CR species will enable the nature of antigenic cross-reactivity to be determined. In this respect, it is somewhat surprising that Bla g 2, the aspartic protease, appears to be expressed only in *B. germanica*, since this enzyme would be expected to occur in both species.

In conclusion, molecular cloning studies have determined the primary structures of several important CR allergens and allowed expression of these allergens in different species to be compared. Manipulation of these sequenc-

es into high-level expression vectors will enable large quantities of recombinant allergens to be produced for clinical and immunologic studies. Studies on the biologic function of CR allergens may also reveal new pathways or mechanisms which could be used to control cockroach infestation in houses.

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