Isolation and Characterization of Clones Encoding Cockroach Allergens

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Key Words

Allergen

German cockroach cDNA IgE binding

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The role of the cockroach as an etiologic agent of respiratory allergic disorders is well documented [1–4]. Cockroach extracts, including cast skins, egg shells, and fecal material [5–7] have been shown to contain several major and minor allergens [8–10]. Despite the fact that positive skin test reactivity to cockroach is second only to house dust, the identification of the clinically relevant antigens and an understanding of the immunobiology of asthmatic reactions is just beginning [11]. This lack of information is partly due to the difficulty involved in purifying allergens from extracts for detailed characterization. Cloning of allergens provides an efficient means of producing pure poly-peptides which, in their native source, form complex mixtures and are often represented in very small amounts.

Recently, the identification and characterization of a variety of inhalant allergens has been accomplished by first purifying an antigenic/allergenic protein and then isolating its recombinant clone. However, since it appears that there are many different allergen sources involved in allergenic asthma, we wanted to explore a cockroach cDNA expression library, screened with serum IgE from cockroach hypersensitive patients, to rapidly isolate many different cockroach allergen clones. These clones could then be used to produce large quantities of pure allergens for characterization. RNA isolated from the German cockroach, *Blattella germanica*, was used to construct an expression library. Numerous clones were isolated after screening 106 plaques with serum IgE from a cockroach-sensitive patient. A subset of phage positive for IgE binding were plaque-purified to homogeneity and tested for their ability to react with serum IgE from cockroach and non-cockroach-sensitive individuals. All of the selected clones were intensely positive when incubated with cockroach-sensitive patient IgE. In contrast, the same clones did not react with control serum IgE.

Recombinant cockroach proteins expressed from a variety of selected clones were analyzed by SDS-PAGE/IgE Western blot to determine their approximate molecular weight. Nineteen clones produced cockroach-specific allergens varying in size from greater than 100 to less than 40 kD. Control blots with non-cockroach-sensitive serum had no detectable IgE binding. These results show that we have isolated numerous clones capable of producing IgE-recognizable antigens specific to patients who are cockroach hypersensitive.

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A recombinant cockroach protein expressed by clone 1 Ala in *Escherichia coli* XL-Blue cells was tested by SDS-Page/IgE Western blot analysis against serum IgE from fourteen patients with documented cockroach hypersensitivity, to address the frequency of reactivity with this allergen. Of the 14 patient sera tested, 8 (57%) had IgE which recognized this recombinant cockroach allergen (table 1). Failure of individuals to recognize this recombinant protein could be due to their requiring a conformational epitope that is not conserved in the recombinant protein or a linear epitope missing from clone lAla

These results are significant in what they reveal about the allergenic epitopes responsible for this reaction. Because these recombinant allergens are expressed in procaryotic cells they are nonglycosylated; therefore, carbohydrate cannot play a role in the recognition of these epitopes. In addition, the allergens bind serum IgE after electrophoresis on denaturing SDS-polyacrylamide gels indicating that the responsible epitopes are linear amino acid sequences not requiring secondary structure.

In summary, the cockroach gene we have isolated is capable of producing a protein product (> 80 kD) in procaryotic cells that is recognized by serum IgE from 57% of the patients' serum tested and therefore can be potentially classified as a major allergen [12, 13]. The use of recombinant cockroach allergens that retain IgE-recognizable epitopes may provide the basis for improving diagnosis and therapy of persons with cockroach hypersensitivity.

Table 1. Patient IgE binding to cockroach clone lAla

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