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Defined Epitopes: In vivo and in vitro Studies Using Recombinant Allergens

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Key Words

Allergen
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Abstract

Background: The aim of the studies was to investigate the biologic activity of recombinant cockroach and mite allergens and their potential for use in diagnosis and treatment of allergic disease. **Methods:** Cockroach allergens Bla g 2, Bla g 4 and Bla g 5 and mite group 5 allergens were produced in bacterial expression vectors and used for immediate skin and serum IgE antibody tests. **Results:** The cockroach allergens showed very good skin test reactivity in allergic patients, giving positive reactions at 10^{-2} – 10^{-5} µg/ml; controls were negative at 10^0 µg/ml. These reactions correlated with serum IgE antibody results. The prevalence of reactivity to group 5 mite allergens varied with exposure. There was a high prevalence (70%) of sensitization to *Blomia tropicalis* allergen, Blo t 5, among patients from Brazil and Singapore, whereas <20% of patients from Charlottesville, US and Manchester, UK gave positive skin tests to Blo t 5 ($p < 0.001$). **Conclusions:** The results show that recombinant allergens retain biologic activity and suggest that cocktails of two to four recombinant allergens could be used for diagnostic or therapeutic purposes. The phased introduction of recombinant allergens should improve the management of allergic disease.

Introduction

Following the cloning of Der p 1 by Thomas et al. in 1988, there has been tremendous progress in allergen molecular biology, and now most major allergens from pollens, mites, animal danders, cockroaches, fungi and several foods have been cloned and sequenced. The impact of this work has been impressive: over 100 allergen sequences are available in the data bases; the biologic function of many allergens is now known; expression vectors have been developed for production of recombinant allergens, and the antigenic structure of allergens in terms of antibody and T cell recognition is being dissected in many laboratories (table 1) [1]. As a result, it is now realistic to envisage that recombinant allergens will increasingly be used in research studies and,

more importantly, will begin to be assimilated into clinical practice either for diagnostic or for therapeutic purposes. The approach to diagnosis would be to use a cocktail of three to four cloned allergens instead of conventional heterogeneous allergen extracts. This would be dependent on recombinant allergens having a biologic activity similar to their natural counterparts. It would also be possible to use recombinant allergen cocktails for immunotherapy. However, most strategies currently under evaluation in clinical trials involve using T cell peptides derived from allergen sequences, or other strategies using allergens to downregulate T cell responses or to alter T cell phenotype.

Over the past 3 years, we have cloned allergens from *Blattella germanica* (German cockroach; Bla g 2, Bla g 4, Bla g 5) and from the dust mite, *Blomia tropicalis* (Blo t 5)

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[2–4]. This paper describes the use of these recombinant allergens to assess sensitization for IgE antibody responses and the potential for using these reagents for diagnosis and treatment.

Materials and Methods

B. germanica Allergens

Recombinant Bla g 4 was produced as a fusion protein in the pGEX expression vector and purified by glutathione affinity chromatography and thrombin cleavage; rBla g 5 (a glutathione transferase) was produced in pET21a and purified in a single step by affinity chromatography over glutathione [3]. Although Bla g 2 (an aspartic protease) had been cloned, attempts to produce immunoreactive recombinant allergen in bacteria or yeast expression systems have so far been unsuccessful. Therefore, natural Bla g 2 was purified by monoclonal affinity chromatography and HPLC [2]. All allergens were of >95% purity as determined by silver-stained SDS-PAGE analysis.

B. tropicalis Allergen, Blo t 5

This allergen was cloned from a *B. tropicalis* cDNA library and showed 40% homology to Der p 5 from *Dermatophagoides pteronyssinus*. Recombinant Blo t 5 was produced in the pGEX vector as described [3].

Human Subjects and Skin Testing

Protocols for skin testing using recombinant allergens were approved by the University of Virginia Human Subjects Investigation Committee. Intradermal skin tests were carried out using tenfold dilutions of allergen extract or of recombinant allergens from 10^{-6} to 10^{-1} µg/ml, and a wheal of >8 × 8 mm at 15 min was regarded as positive.

Results and Discussion

Skin tests to cockroach allergens were carried out on selected adult patients who presented to the University of Virginia Allergy Clinic with asthma. These patients gave strongly positive skin tests to commercial *B. germanica* extract (10^{-5} – 10^{-6} µg/ml), and they also gave good responses to either nBla g 2, rBla g 4 or rBla g 5 (table 2). Some patients (e.g. J.S., S.E.) gave positive skin tests to all three purified allergens, whereas others responded to only one or two allergens (e.g. M.B., S.T.). There was no evidence for nonspecific reactivity to the recombinant allergens or to a GST vector control in nonallergic individuals. The prevalence of IgE antibodies to four cockroach allergens (Bla g 1, Bla g 2, Bla g 4, Bla g 5) was assessed in more than 70 sera from cockroach-allergic patients by the enzyme immunoassay. The results showed that measuring IgE antibodies to each of these allergens could identify >95% of patients with IgE antibodies to *B. germanica*.

Table 1. Impact of molecular biology on allergen research and clinical practice

Identification and cloning of allergens
Sequence homology searches – biologic function
Production of recombinant allergens
Identification of antigenic determinants
Site-directed mutagenesis (IgE antibodies)
Derivation of T cell clones and epitope mapping
Analysis of allergen-specific immune response
Molecular modeling/three-dimensional structure
Development of new diagnostic and therapeutic products

Table 2. Biologic activity of recombinant cockroach allergens

	Intradermal skin test titer			
	<i>B. germanica</i>	nBla g 2	rBla g 4	rBla g 5
Patients				
J.S.	$10^{-5.5}$	10^{-3}	10^{-3}	10^{-4}
S.E.	$10^{-5.5}$	$10^{-2.5}$	10^{-5}	10^{-3}
T.B.	10^{-5}	n.d.	10^{-5}	10^{-3}
R.M.	$10^{-5.5}$	10^{-3}	10^{-3}	10^{-1}
M.B.	10^{-6}	$10^{-2.5}$	>1	n.d.
S.T.	$10^{-5.5}$	10^{-1}	>1	>1
Controls (n = 4)	> 10^{-2}	>10	>10	>10

Skin tests to *B. tropicalis* and *D. pteronyssinus* allergens have been carried out as part of a multicenter international study to evaluate allergenic cross-reactivity between these species. The study was designed to compare IgE responses to the two mite species and to the recombinant group 5 allergens in areas where patients were either exposed to both mites (Brazil, Singapore) or only to *D. pteronyssinus* (Charlottesville, US and Manchester, UK). In Brazil and Singapore, 60–70% of mite allergic patients gave positive skin tests or had serum IgE antibodies to rBlo t 5. In contrast, the prevalence of sensitization was <20% in patients from Charlottesville or Manchester ($p < 0.001$). In keeping with previous studies, the prevalence of sensitization to rDer p 5 was 40–50% amongst each group of patients [5]. Interestingly, 60% of 'unexposed' patients from Charlottesville or Manchester gave positive skin tests to both *D. pteronyssinus* and *B. tropicalis*, suggesting that these species also produce cross-reacting allergens. In Brazil and Singapore, the magnitude of skin test responses to rDer p 5 and rBlo t 5 (in terms of intradermal titer) was very similar. However, among the few patients from Charlottesville who gave positive skin tests to Blo t 5, the titer was 3–4 logs lower than to Der p 5.

Our results on the biologic activity of recombinant cockroach and mite allergens suggest that it will be possible to use selected recombinant allergens for clinical and investigative purposes. Cockroach appears to be a multiallergen system where a cocktail of as many as four allergens could be required for full diagnostic sensitivity. We have recently shown that both Bla g 2 and Bla g 4 can induce proliferative T cell responses in some cockroach-allergic patients, suggesting that these allergens could be candidates for T-cell-based immunotherapies. Blo t 5 appears to be a major allergen in areas where *B. tropicalis* is endemic. We have also cloned another allergen (Bt8, troponin) from the *B. tropicalis* cDNA library. Our results make it clear that Blo t 5 alone would be insufficient as an indicator of sensitization to *B. tropicalis*, but a combination with another cloned allergen could be enough for most clinical purposes. *B. tropicalis* extract is not yet available commercially and, therefore, the recombinant allergens may well serve as better diagnostic reagents.

The results suggest that recombinant allergens will increasingly be able to be used in allergy practice. Clearly, the biologic activity of each recombinant allergen will need to be compared with their natural counterparts (where possible) in a large population of allergic patients, and controlled clinical trials will be necessary to evaluate each allergen on a case-by-case basis. We have also shown with Der p 2 that the biologic activity of allergens can be modified by mutagenesis and that variants with markedly reduced IgE-antibody-binding activity can be generated [6–8]. Such variants may be useful in immunotherapy because the precise amino acid substitutions can be defined and their effects on both antibody and T cell responses can be evaluated. The introduction of recombinant allergens for diagnostic and therapeutic purposes should significantly improve the management of allergic diseases.

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