

Four Novel Recombinant Peanut Allergens: More Information, More Problems

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Key Words

Ara h 4 · Peanut profilin (Ara h 5) · Ara h 6 · Ara h 7 ·
Codon usage

Introduction

Peanuts are the cause of a number of different allergic symptoms with differing degrees of severity. These clinical symptoms are the oral allergy syndrome, atopic dermatitis, asthma, diarrhoea and anaphylactic reactions. It was recently shown that in peach allergy, the oral allergy syndrome is caused by the Bet v 1-analogous allergen, whereas severe clinical reactions are induced by the lipid transfer proteins [1]. Since peanuts contain several allergenic components, the not inept question arises whether these components are associated with distinct clinical symptoms or the severity of the clinical reaction. If so, this would be a starting point for improving the in vitro diagnostics of peanut allergy based on single allergenic components. Furthermore, the identification of 'anaphylaxis-inducing and -eliciting allergens' would put the relevant allergens in focus for designing safe immunotherapeutic drugs.

Results and Discussion

A prerequisite to answering these questions is the identification and characterization of these components on a molecular level. An ideal method to clone the allergenic entities completely is the phage display system where patients' IgE is the selection and enrichment agent [2]. In this system, the presented allergen and its gene-containing phages are selected by patients' IgE using the panning method. The selected phages are multiplied in *Escherichia coli* and in the next cycle selected and enriched and so on. After five cycles, we were able to clone six different allergens [3]. Two of them were the well-known major allergens of peanut, Ara h 1 and Ara h 2. Ara h 4, Ara h 5 (profilin), Ara h 6 and Ara h 7 are first described in their structure deduced from the DNA sequence. Ara h 4, Ara h 6 and Ara h 7 show significant sequence similarities to seed storage proteins, whereby Ara h 6 and 7 belong to the conglutin family. They are not isoforms of Ara h 2 but independent gene products. Ara h 3 is an isoform of Ara h 4 with 91% identity, which was registered in the database and announced to the nomenclature subcommittee of the IUIS before Ara h 3 [3]. Testing the IgE reactivity of peanut-allergic patients by Western blotting, we found that Ara h 1 was recognized by 65%, Ara h 2 by 85%, Ara

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Table 1. The different codon usage of peanut allergens and *E. coli*

Amino acid codon	Ara h 1	Ara h 2	Ara h 4	Ara h 5	Ara h 6	Ara h 7	<i>E. coli</i>
Arginine AGA, AGG	5.4	9.6	8.1	0.8	8.1	8.6	0.4
Isoleucine AUA	0.8	0.6	0.8	0.0	2.4	0.0	0.5
Leucine CUA	0.0	0.6	0.2	0.0	0.0	0.0	0.4
Proline CCC	1.5	0.6	1.1	0.8	1.6	2.2	0.5

Table 2. Clinical data of 8 out of 40 peanut-allergic patients

Patient	Age years	CAP/peanut-specific IgE/kU/l	CAP, others	Total IgE/kU/l	Symptoms	Further allergies	Specific IgE reactivity
JG3	25	6/>100	3, birch pollen 3, hazelnut 4, grass pollen	3,693	OAS, D, R, A, N, U shock (aerosol reaction)	tree/grass pollen HDM, latex	Ara h 1, 2, 4, 6, 7
HF4	36	5/63.2	4, birch pollen 2, hazelnut 3, grass pollen	329	OAS, A, U, N, shock	none	Ara h 1, 2, 4, 6, 7
JB5	22	4/32	5, birch pollen 3, hazelnut 4, grass pollen	3,961	OAS	pollinosis (in May), asthma	Ara h 1, 2, 4, 7
23	25	3/13.7	3, birch pollen	93	OAS, D, R, A, N, shock	none	Ara h 1, 2, 4, 6, 7
26	25	5/81.4	2, soybean	438	OAS, D, R, A, N shock	none	Ara h 1, 2, 4, 6, 7
28	18	5/80.7	6, birch pollen 5, hazelnut 6, grass pollen	1,696	OAS	tree/grass pollen	Ara h 5
31	12	6/>100	2, grass pollen 2, hazelnut	393	OAS, A, U, shock	none	Ara h 1, 2, 4, 6, 7
SC	9	6/>100	2, birch pollen 3, grass pollen	279	U		Ara h 1, 2, 4, 6, 7

OAS = Oral allergy syndrome; R = rhinitis, A = asthma; N = neurodermatitis; U = urticaria; D = diarrhoea;
AD = atopic dermatitis; HDM = house dust mite.

h 4 by 53%, Ara h 5 by 13%, Ara h 6 by 38% and Ara h 7 by 43% of 40 sera [3]. As antigens we used cell lysates. However, apart from the peanut profilin (Ara h 5), we have experienced difficulties in expressing the other recombinant peanut allergens with a satisfactory yield for further experimental work which needs purified recombinant allergens.

With regard to the poor expression yields of recombinant Ara h 1, we recently discussed the different codon usage of *E. coli* and peanut in relation to arginine as the cause [4]. The different codon usage with regard to argi-

nine and other amino acids of peanut and *E. coli* is shown in table 1. With respect to Ara h 1, we tried to overcome this problem by cloning the respective allergen in *Pichia pastoris*, but with minor success [5].

The idea of mastering the codon usage problem by providing rare tRNAs in the expression system has now been realized by the development of BL21 (DE3) Codon Plus RIL™ cells (Stratagene). In these cells, we were able to express Ara h 2 [6], Ara h 1 and Ara h 6 [7] with excellent yields. Thus, we found an elegant way to overcome the expression problems of recombinant peanut allergens in

E. coli. That opened the way to investigating the question whether certain peanut allergens are associated with clinical symptoms and the severity of the clinical reactions. Out of 40 patients, 8 were chosen and their clinical data is shown in table 2. At first glance, there is no difference in the IgE reaction pattern of shock patients and patients with oral allergy syndrome to recombinant peanut allergens. The fact that Ara h 6 is detected by the IgE of patients with shock symptoms and urticaria but not by the IgE of patients with an isolated oral allergy syndrome may be an indication that Ara h 6 is a candidate for association with severe clinical reactions. This is underlined by the findings of Sellers et al. [8], who were able to show that Ara h 6 (the 14-kD component) is resistant to pepsin deg-

radation. Whether the measurement of the intensity of IgE reactivity to certain peanut allergens by binding assays provides clearer associations between clinical symptoms and these allergens is now under investigation. If the outcome of this study should be insufficient, then we will try to improve peanut allergy diagnosis on the basis of allergen substructures, e.g. linear and conformational epitopes.

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