

Review article Series II

Peanut allergens

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Immediate hypersensitivity reactions to foods occur in 6–8% of children and about 1% of adults (1–3). The ingestion of peanuts is a major cause of serious allergic reactions in both children and adults (3). A recent population survey in the USA suggests that 0.7% of children are allergic to peanuts (4). This hypersensitivity reaction often starts early in childhood and continues throughout life. This is in contrast to other childhood food allergies such as those to cow's milk and eggs, which generally resolve spontaneously with age (2). A recent study found that peanut-allergic patients had their initial reaction at a mean age of 22 months (5). Additionally, for unknown reasons, peanut allergy is more often associated with fatal food-induced anaphylaxis than any other food allergy (6–8). Avoidance is the only effective means of dealing with food allergy. The current use of peanuts and peanut byproducts as supplements in many different types of foods makes accidental ingestion almost inevitable. In a 5-year period, over 50% of patients experienced at least one accidental ingestion (4).

Food hypersensitivity reactions occur shortly after contact of an allergen with specific IgE antibodies already bound to mast cells. The cross-linking of allergen-specific IgE by the respective allergen stimulates mast cells to release histamine, heparin, and other mediators responsible for the clinical symptoms observed. As can be seen, the allergens play a vital role in this allergic process. The identification and characterization of the peanut allergens is important if improved diagnostic and therapeutic capabilities are to be developed. Thus, the prevalence and chronic nature of peanut allergy, the potential severity of the allergic reac-

tion, and the widespread use of peanuts in consumer foods necessitates a better understanding of this allergic response.

Background

Several previous studies have examined the nature and location of the multiple allergens in peanuts (9, 10). Several peanut varieties are grown in the USA and throughout the world, including the Virginia, the Spanish, and the runner. These studies showed the allergenic portion of the peanut to be in the protein of the cotyledon, a feature which does not differ significantly among the different varieties of peanuts (11). Peanut-1 was one of the first peanut allergens identified (12) with two major bands at 20 and 30 kDa. In a separate study, investigators demonstrated by RAST inhibition that Con A-reactive glycoproteins bound IgE in >50% of their patients with peanut hypersensitivity (13). More recent studies have shown that some of the seed storage proteins in the plant are the major allergens. Three of the major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, are abundant proteins that belong to the vicilin, conglutin, and glycinin families of seed storage proteins, respectively (Table 1). Ara h 1 and Ara h 2 are recognized by IgE from greater than 95% of peanut-sensitive patients (14–17). The Ara h 3 protein has just recently been identified and appears to be recognized by IgE from a subpopulation of peanut-sensitive patients. These allergens have now been relatively well characterized, cloned, and sequenced, and their IgE-binding epitopes and T-cell epitopes partially identified.

Table 1. Amino-acid sequence analysis of purified peanut allergens

Allergen	MW ¹	Determined amino-acid sequence ²	Sequence homology ³
Ara h 1	65 kDa	I) I-F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K II) K-G-S-E-E-E-G-D-I-T-N-P-I-N-L-R III) N-N-P-F-Y-F-P-S-R-R	Vicilins
Ara h 2	17 kDa	I) X-Q-Q-W-E-L-Q-G-D-R-R-Q-S-Q-L-E-R II) A-N-L-R-P-C-E-Q-H-L-M-Q-K	Conglutin
Ara h 3	14 kDa	I) I-S-F-R-Q-Q-P-E-E-N-A-X-Q-F-Q-R-L-N-A-Q-R-P-D	Glycinin

¹Molecular weights were estimated from SDS-PAGE gels and are only approximations.

²Amino-acid sequences are represented as one-letter amino-acid code and were determined either from amino terminus of isolated protein or from peptides produced by tryptic digestion of purified protein.

³Sequence homology was determined by performing database searches with determined amino-acid sequence. Indicated proteins had highest homology with peanut allergen.

Peanut allergens

Ara h 1

Ara h 1 is a 64.5-kDa glycoprotein recognized by >95% of patients with peanut hypersensitivity. The Ara h 1 allergen was initially identified by IgE-immunoblot analysis with pooled serum from peanut-sensitive patients. Utilizing amino terminal sequence information from the purified allergen and molecular biology techniques, we isolated a full-length cDNA clone encoding this allergen. After characterization of the cDNA clone and comparison of the derived Ara h 1 amino-acid sequence with the plant protein database, it was determined that Ara h 1 belonged to the vicilin family of seed storage proteins. To determine the specific, linear IgE-binding epitopes of this allergen, overlapping peptides representing the entire Ara h 1 primary amino-acid sequence were synthesized on a cellulose membrane containing free hydroxyl groups, Fmoc-amino acids (Genesis Biotechnology) being used. After incubation with serum IgE from patients with peanut hypersensitivity, 23 different linear IgE-binding epitopes were identified, located throughout the length of the Ara h 1 protein. Four of the peptides appeared to be immunodominant IgE-binding epitopes in that they were recognized by >80% of the patients and bound more IgE than any of the other Ara h 1 epitopes.

Mutational analysis of the immunodominant epitopes revealed that single amino-acid changes, particularly in the center of each epitope, significantly reduced peanut-specific IgE binding. Hydrophobic residues appeared to be the most critical for IgE binding. For clarification of the interaction of this allergen with IgE, an homology-based model of Ara h 1's tertiary structure was constructed (18). The position of the IgE-binding epitopes on the tertiary structure of this allergen revealed that they were clustered into two main

regions, despite their even distribution throughout the primary sequence. Subsequent to that, we utilized a reproducible immunofluorescence assay to show that, like other vicilins, Ara h 1 forms a stable homotrimer. Interestingly, when the IgE-binding epitopes are placed on this structure, they all cluster at the contact points where each monomer is joined to the other. This structure may explain in part the extreme allergenicity exhibited by this molecule. Currently, the possibility of producing a hypoallergenic protein is being explored in our laboratory. Utilizing the information that each of the IgE-binding epitopes can be mutated to a non-IgE-binding site by the alteration of a single amino acid, we are performing site-directed mutagenesis on the Ara h 1 gene. After producing the modified recombinant protein, we will test it for IgE-binding activity by immunoblot analysis and skin prick test. Successful results from these experiments may lead to new immunotherapeutic approaches to allergic disease.

Ara h 2

This glycoprotein of 17.5 kDa was initially identified from crude peanut extracts. With N-terminal amino-acid sequencing data from purified Ara h 2, oligonucleotide primers were synthesized and used to identify a clone (741 bp) from a peanut cDNA library (17). This clone could encode a 17.5-kDa protein with homology to the conglutin family of seed storage proteins (Table 2). As in work done on Ara h 1, the linear IgE-binding epitopes were identified. Ten IgE-binding epitopes were found to be distributed throughout the length of the Ara h 2 protein. Of the amino acids represented in the epitopes, 63% were either polar uncharged or apolar residues. When each set of the 10 peptides were probed individually with serum from patients with peanut hypersensitivity, all of the patients recognized multiple epitopes. Three epitopes (aa

Table 2. Ara h 2 amino-acid sequence

LTILVALALFLAAHASARQQWELQGDRRCSQLERA-37
NLRPCEQHLMQKIQRDEDSYERDPYSPSQDPYSPSPY-74
DRRGAGSSQHQRCCNELNEFENNQRMCCEALQQIME-111
NQSDRLQGRQQEQKFRELRLNPQQCGLRAPQRCDLD-148
VESGGRDRY-157

Ara h 2 amino-acid sequence is represented as single-letter amino-acid code. Alanines were substituted for wild-type residue at position indicated in Fig. 1.

27–36, aa 57–66, and aa 65–74) were recognized by all patients, indicating that they represent the immunodominant epitopes of Ara h 2. Mutational analysis of the Ara h 2 epitopes indicated that single amino-acid changes, particularly in the center of each epitope, resulted in loss of peanut-specific IgE binding. The Ara h 2 gene was mutagenized so that the individual amino-acid residues within the immunodominant epitopes were changed to alanine residues, and the recombinant version of this modified protein was produced. Immunoblot analysis of this modified recombinant protein using serum IgE from peanut-sensitive patients showed that the mutagenized version of this allergen bound significantly less IgE than the wild-type version of this recombinant protein (Fig. 1).

Similarly, T-cell-epitope mapping was done with short-term T-cell lines from patients with peanut hypersensitivity. Overlapping peptides were generated and then utilized in antigen-specific stimulation assays. Four immunodominant regions distributed throughout the linear sequence could be identified. These regions represent amino-acid residues 19–28, 45–53, 96–114, and 131–139. With the exception of one T-cell epitope (aa 19–28), all the other T-cell epitopes mapped to different locations than the immunodominant IgE-binding epitopes. This is an important finding because it will allow the development of an immunotherapeutic reagent where IgE-binding sites have been altered so they no longer bind IgE, but T-cell binding sites remain intact.

Ara h 3

Previous studies have shown significant *in vitro* antibody cross-reactivity among peanuts and soybeans, while clinical reactivity is often specific for

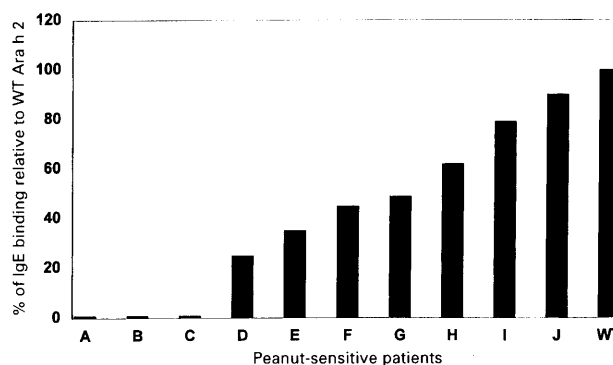


Fig. 1. IgE binding to mutant Ara h 2.

either one food or the other. To identify peanut-specific allergens, serum IgE from patients with documented peanut hypersensitivity reactions was absorbed with soybeans to remove any cross-reacting antibodies (19). A 14-kDa protein (Ara h 3) was identified by these soy-adsorbed sera. Subsequent amino-acid sequence analysis of this protein revealed a high homology to a family of proteins called glycinins. Glycinins represent a class of seed storage proteins that are first synthesized as precursor proteins of ~60 kDa and are then proteolytically cleaved into 40- and 20-kDa proteins. A peanut cDNA clone encoding the full-length protein (60 kDa) was isolated and characterized. The 14-kDa protein identified by the soy-adsorbed sera from immunoblot analysis of crude peanut extracts represented the amino terminal end of the peanut glycinin. Whether this 14-kDa protein represents the natural product of the cleavage of the peanut glycinin or a breakdown product resulting from nonspecific proteolytic cleavage remains to be determined. As in our previous studies, overlapping peptides representing the entire amino-acid sequence of this peanut glycinin will be generated to determine the IgE-binding epitopes on the Ara h 3 allergen.

Other possible peanut allergens

One of our hypotheses is that IgE antibodies in peanut-allergic patients that cross-react with soybean proteins are not responsible for the allergic reaction to peanuts, since most peanut-allergic children can ingest soybeans with no evidence of clinical reactivity (20). Utilizing soy-adsorbed sera from peanut-allergic patients and glycine- and tricine-SDS-PAGE, we determined that less than 10 of the more than 30 possible peanut proteins were likely to be clinically relevant. All of the proteins of less than 20 kDa isolated in this manner were identified either by N-terminal sequencing or tryptic digestion followed by N-terminal sequencing of

individual tryptic peptides. Each of these allergens was either a fragment or an isoform of Ara 1, 2, or 3. Whether protein fragments identified by immunoblotting are expressed naturally in the peanut or represent cleaved products due to endogenous proteases is not clear. Nevertheless, Ara h 1, 2, and 3 appear to constitute the major clinically relevant peanut allergens.

The potential role of carbohydrate in allergenicity

Most food allergens are glycoproteins. While there has been no obvious sequence similarity noted between the different IgE-binding epitopes of the allergenic proteins, a common characteristic is that most of the proteins contain N-linked oligosaccharides having a β 1,2-linked xylose attached to the β -linked mannose of the core structure (21). This structure is unique in plants and invertebrates and absent in higher eukaryotic organisms. In wheat and barley glycoproteins, xylose appears to be important for allergenicity, since those proteins containing β 1,2-xylose were more reactive *in vivo* and *in vitro* than deglycosylated proteins (22). Furthermore, IgE from wheat- or barley-allergic patients recognized an endo-Lys C peptide containing the glycan, and this recognition was lost upon deglycosylation (23). With antibodies directed against the β 1,2-linked xylose, the Ara h 1 and Ara h 2 peanut allergens have also been shown to contain this unique carbohydrate structure. The enzyme that adds xylose to these N-linked glycoproteins is the β 1,2-xylosyltransferase. This enzyme has been purified from soybean cells and its properties have been described (24). The cDNA encoding this enzyme has just recently been isolated, and the recombinant protein remains to be characterized. Whether this unique carbohydrate structure plays an active role in peanut allergenicity as an IgE-binding domain or a structure that targets the protein to specific cellular organelles, or simply acts to protect the allergen from digestion, remains to be determined. However, with these tools, it should now be possible to reconstitute the entire peanut allergen to determine the role each plays either in sensitizing an individual or in the clinical reaction observed in an individual already sensitized to peanuts.

Summary

The development of an IgE response to an allergen involves a series of interactions between T cells and B cells. B cells bearing appropriate antigen-specific surface immunoglobulins interact with proliferating allergen-specific T cells, leading to isotype

switching and the generation of antigen-specific IgE. The antigen-specific IgE then binds to the Fc ϵ RI receptors of mast cells and basophils. Because antigen-specific IgE plays such a critical role in the pathogenesis of allergic disease, determination of allergen-specific, IgE-binding epitopes is an important first step toward a better understanding of this complex disease process. Studies defining the peanut allergens should now allow more specific research to be done on improved diagnostic methods for peanut hypersensitivity, new immunotherapeutic approaches for this chronic and often severe disease, and development of hypoallergenic or less sensitizing plants.

Immediate hypersensitivity reactions to foods are mediated through the interaction of IgE with a specific food protein. While specific IgE-binding epitopes from the major allergens of cow's milk (25), codfish (26), hazelnut (27), soybeans (28), and shrimp (29) have been elucidated, there have been few, if any, common characteristics found in these binding sites. Our work on the IgE-binding sites of Ara h 1, 2, and 3 indicates that there are no common amino-acid sequence motifs shared by these epitopes. However, we have determined that in the IgE-binding epitopes of these allergens the hydrophobic amino-acid residues appear to play a critical role in IgE binding. The observation that alteration of a single amino acid leads to the loss of IgE binding in a population of peanut-sensitive individuals is significant because it suggests that while each patient may display a polyclonal IgE reaction to a particular allergen (16, 17), IgE from different patients recognize the same epitope and must interact with that epitope in a similar fashion. Besides after the finding that many epitopes in each of the different peanut allergens contained more than one residue critical for IgE binding, it was also determined that more than one residue type (ala or met) could be substituted at certain positions in an epitope with similar results. This information may allow the design of a hypoallergenic protein that would be effective in blunting allergic reactions for a population of peanut-sensitive individuals. Furthermore, a peanut from which the IgE-binding epitopes of the major allergens have been removed may prevent the development of peanut hypersensitivity in individuals genetically predisposed.

The characteristics that have been attributed to allergenic proteins include their abundance in the food source, their resistance to food processing, and their stability to digestion by the gastrointestinal tract (30–32). The major allergens of foods – in particular, the Ara h 1 peanut allergen – have been shown to survive intact most food-processing methods and to be stable to digestion in *in vitro*

systems designed to mimic the gastrointestinal tract. Our observations on the tertiary structure of the Ara h 1 monomer and the determination that this protein readily forms a trimeric complex may help to determine why this protein is allergenic. While there are numerous protease digestion sites throughout the length of this protein, the structure may be so compact that potential cleavage sites are inaccessible until the protein is denatured. The physical properties of the Ara h 1 molecule and the other peanut allergens may help to explain the extreme allergenicity exhibited by peanut proteins.

The only therapeutic option currently available for the prevention of a peanut hypersensitivity reaction is food avoidance. Unfortunately, for a ubiquitous food such as peanut, the possibility of an inadvertent ingestion is great. Interestingly, most of the peanut allergens identified to date, including Ara h 1, 2, and 3, have sequence homology with proteins in other plants. This information may help to begin to explain the cross-reacting IgE antibodies to other legumes that are found in the sera of patients that manifest clinical symptoms to only one member of the legume family (33). Certainly, the elucidation of the position of the Ara h 1-binding epitopes clustered on the surface of the molecule may enable us to understand why these regions elicit the clinical symptoms associated with peanut hypersensitivity. Perhaps the presentation of multiple, clustered epitopes to mast cells results in a more efficient and dramatic release of mediators, resulting, in turn, in the more severe clinical symptoms observed in patients with peanut hypersensitivity. Current work is exploring this possibility by comparing the IgE-binding epitopes and tertiary structures of other legume allergens.

Taken in total, these studies suggest that an altered Ara h 1, 2, or 3 gene could be developed to replace its allergenic homologue in the peanut genome, thus blunting allergic reactions in sensitive individuals who inadvertently ingest this food. Since these gene products are an abundant and integral seed storage protein, it would be necessary for the altered protein to retain as much of its native function, properties, and three-dimensional structure as possible. The information gained from these studies on the peanut allergens indicates that development of hypoallergenic seed storage proteins may be feasible. Additionally, a less allergenic peanut protein with the immunodominant IgE epitopes mutated, rather than the current peanut protein as we know it, may prove to be beneficial in feeding the at-risk group of atopic individuals. However, the effect that altering critical amino acids within each of the IgE-binding epitopes has on the properties of the seed storage proteins is currently unknown. In view of the widespread use

of peanuts in consumer foods and the potential risk this poses to individuals genetically predisposed to developing peanut allergy and to the health of individuals already peanut sensitive, this approach is currently being explored in our laboratories.

References

1. Jansen JJ, Kardinaal AFM, Huijber G, Vlieg-Boestra BJ, Martens BP, Ockhuizen T. Prevalence of food allergy and intolerance in the adult Dutch population. *J Allergy Clin Immunol* 1994;93:446-56.
2. Burks AW, Sampson HA. Food allergies in children. *Curr Prob Pediatr* 1993;23:230-52.
3. Bock SA. Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. *Pediatrics* 1987;79:683-8.
4. Sicherer SH, Munoz-Furlong A, Burks AW, Sampson H. Prevalence of peanut and tree nut allergy in the United States of America. *N Engl J Med* (Submitted 1998).
5. Sicherer SH, Burks AW, Sampson H. Clinical features of acute allergic reactions to peanut and tree nuts in children. *J Pediatr* 1998 (in press).
6. Fries JH. Peanuts: allergic and other untoward reactions. *Ann Allergy* 1982;48:220-6.
7. Ewan P. Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *BMJ* 1996;312:1074-8.
8. Sampson HA, Mendelson LM, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992;327:380-4.
9. Bush RK, Taylor SL, Nordlee JA. Peanut sensitivity. *Allergy Proc* 1989;10:261-4.
10. Gillespie DN, Nakajima S, Gleich GJ. Detection of allergy to nuts by the radioallergosorbent test. *J Allergy Clin Immunol* 1976;57:302-9.
11. Taylor SL, Busse WW, Sachs MI, Parker JL, Yunginger JW. Peanut oil is not allergenic to peanut sensitive individuals. *J Allergy Clin Immunol* 1981;68:372-5.
12. Sachs MI, Jones RT, Yunginger JW. Isolation and partial characterization of a major peanut allergen. *J Allergy Clin Immunol* 1981;67:27-34.
13. Barnett D, Howden MEH, Bonham B, Burley RW. Aspects of legume allergy research. *Proc Sydney Allergy Group* 1985;4:104-18.
14. Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien T. Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* 1991;88:172-9.
15. Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien T, Helm RM. Identification and characterization of a second major peanut allergen, Ara h II, utilizing the sera of patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* 1992;90:962-9.
16. Burks AW, Cockrell G, Stanley JS, Helm RM, Bannon GA. Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. *J Clin Invest* 1995;96:1715-21.
17. Stanley JS, King N, Burks AW, et al. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch Biochem Biophys* 1997;342:244-53.
18. Shin DS, Compadre CM, Sampson H, et al. Biochemical and structural analysis of the IgE binding sites on Ara h 1, an abundant and highly allergenic peanut protein. *J Biol Chem* 1998 (in press).

19. Eigenman PA, Burks AW, Bannon GA, Sampson HA. Identification of unique peanut and soy allergens in sera adsorbed with cross-reacting antibodies. *J Allergy Clin Immunol* 1996;98:969-78.
20. Sampson HA, Huang SK, Burks AW, Bannon GA, Buckley N. Characterization of major peanut allergens. *J Allergy Clin Immunol* 1998;101:240A.
21. Calkhoven PG, Aalberse M, Koshte VL, Pos O, Oei HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables, and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987;42:382-90.
22. Tezuka K, Hayashi M, Ishihara H, Akazawa T, Takahashi N. Studies on synthetic pathway of xylose-containing *N*-linked oligosaccharides deduced from substrate specificities of the processing enzymes in sycamore cells (*Acer pseudoplatanus* L.). *Eur J Biochem* 1992;203:401-13.
23. Garcia-Casado G, Sanchez R, Chrispeels MJ, Armentia A, Salcedo G, Gomez L. Role of complex asparagine-linked glycans in the allergenicity of plant glycoproteins. *Glycobiology* 1996;6:471-7.
24. Zeng Y, Bannon GA, Thomas VH, Rice K, Drake R, Elbein A. Purification and specificity of β 1,2-xylosyltransferase, an enzyme that contributes to the allergenicity of some plant proteins. *J Biol Chem* 1997;272:31340-7.
25. Ball G, Shelton MJ, Walsh BJ, Hill DJ, Hoskins CS, Howden ME. A major continuous allergenic epitope of bovine beta-lactoglobulin recognized by human IgE binding. *Clin Exp Allergy* 1994;24:758-64.
26. Aas K, Elsayed S. Physico-chemical properties and specific activity of a purified allergen (codfish). *Dev Biol Stand* 1975;29:90-8.
27. Elsayed S, Holen E, Dybenal T. Synthetic allergenic epitopes from the amino-terminal regions of the major allergens of hazel and birch pollen. *Int Arch Allergy Appl Immunol* 1989;89:410-15.
28. Herian AM, Taylor SL, Bush RK. Identification of soybean allergens by immunoblotting with sera from soy-allergic adults. *Int Arch Allergy Appl Immunol* 1990;92:193-8.
29. Shanti KN, Martin BM, Nagpal S, Metcalfe DD, Rao PV. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE binding epitopes. *J Immunol* 1993;151:5354-63.
30. Astwood JD, Leach JN, Fuchs RL. Stability of food allergens to digestion *in vitro*. *Nat Biotechnol* 1996;14:1269-73.
31. Veiths S, Aulepp H, Becker W-M, Buschmann L. Characterization of labile and stable allergens in foods of plant origin. In: *Food allergies and intolerances*. 1996:130-49.
32. Lehrer SB, Horner WE, Reese G. Why are some proteins allergenic? Implications for biotechnology. *Crit Rev Food Sci Nutr* 1996;36:555-64.
33. Bernhisel-Broadbent J, Taylor S, Sampson HA. Cross-allergenicity in the legume botanical family in children with food hypersensitivity. II. Laboratory correlates. *J Allergy Clin Immunol* 1989;84:701-9.