

Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity

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Peanut allergy is a significant health problem because of the prevalence and potential severity of the allergic reaction. Serum IgE from patients with documented peanut hypersensitivity reactions and overlapping peptides were used to identify the IgE-binding epitopes on the major peanut allergen, Ara h 1. At least twenty-three different linear IgE-binding epitopes, located throughout the length of the Ara h 1 protein, were identified. All of the epitopes were 6–10 amino acids in length, but there was no obvious sequence motif shared by all peptides. Four of the peptides appeared to be immunodominant IgE-binding epitopes in that they were recognized by serum from more than 80% of the patients tested 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 within these peptides had dramatic effects on IgE-binding characteristics. The identification and determination of the IgE-binding capabilities of core amino acids in epitopes on the Ara h 1 protein will make it possible to address the pathophysiologic and immunologic mechanisms regarding peanut hypersensitivity reactions specifically and food hypersensitivity in general.

Keywords: food allergy; IgE-binding epitope; epitope analysis.

Approximately 8% of children and 1–2% of adults have some type of food allergy [1]. Peanuts, fish, tree nuts, and shellfish account for the majority of food hypersensitivity reactions in adults, while peanuts, milk, and eggs cause over 80% of food hypersensitivity reactions in children [2].

Food hypersensitivity reactions occur shortly after contact of a specific allergen with its corresponding IgE antibodies which are bound to mast cells. Allergen-specific IgE, when cross-linked by the respective allergen, activates the mast cells to release histamine, heparin, and other mediators responsible for the clinical symptoms observed. Thus the IgE-binding epitopes of the allergens play an important role in the disease process. Their characterization will provide a better understanding of the human immune response involved in food hypersensitivity reactions. If improved diagnostic and therapeutic capabilities are to be developed it is important to determine the primary structure and frequency of recognition of any IgE-binding epitopes contained within the allergen.

Various studies have shown that the most allergenic portion of the peanut is the protein fraction of the cotyledon [3]. A major allergen found in the cotyledon is the peanut protein, Ara h 1 [4]. The Ara h 1 allergen belongs to the vicilin family of seed storage proteins [5]. This protein is recognized by greater than 90% of peanut-sensitive patients, thus establishing it as an important allergen [4]. The majority of serum IgE recognition of the Ara h 1 allergen appears to be due to epitopes within this protein that are linear amino acid sequences that do not contain significant amounts of carbohydrate [4, 5]. Previous results have demonstrated similarity between the level of IgE binding to re-

combinant Ara h 1 protein and the native form of this allergen when individual patient serum was tested [5]. These results indicated that the recombinant protein could be considered for use in both diagnostic and immunotherapeutic approaches to peanut hypersensitivity.

Because of the prevalence and severity of peanut hypersensitivity reactions in both children and adults, and the role B-cell epitopes play in these reactions, we set out to map and characterize the major IgE epitopes of the Ara h 1 allergen. In this study, we report the primary structure of the Ara h 1 IgE-binding epitopes recognized by peanut hypersensitive individuals. Four epitopes that bound peanut-specific serum IgE from more than 80% of patients tested were identified. The amino acids important to peanut-specific IgE recognition of these epitopes were then determined for the purpose of using them in future diagnostic and immunotherapeutic approaches to this disease.

MATERIALS AND METHODS

Serum IgE. Serum from 15 patients with documented peanut hypersensitivity reactions (mean age, 25 y) was used to identify the Ara h 1 IgE-binding epitopes. Each of these individuals had a positive immediate prick skin test to peanut and either a positive double-blind placebo-controlled food challenge or a convincing history of peanut anaphylaxis (laryngeal edema, severe wheezing, and/or hypotension). Representative individuals with elevated serum IgE levels (who did not have peanut-specific IgE or peanut hypersensitivity) were used as controls in these studies. In some instances, a serum pool was made by mixing equal aliquots of serum IgE from each of the 15 patients with peanut hypersensitivity. This pool was then used in immu-

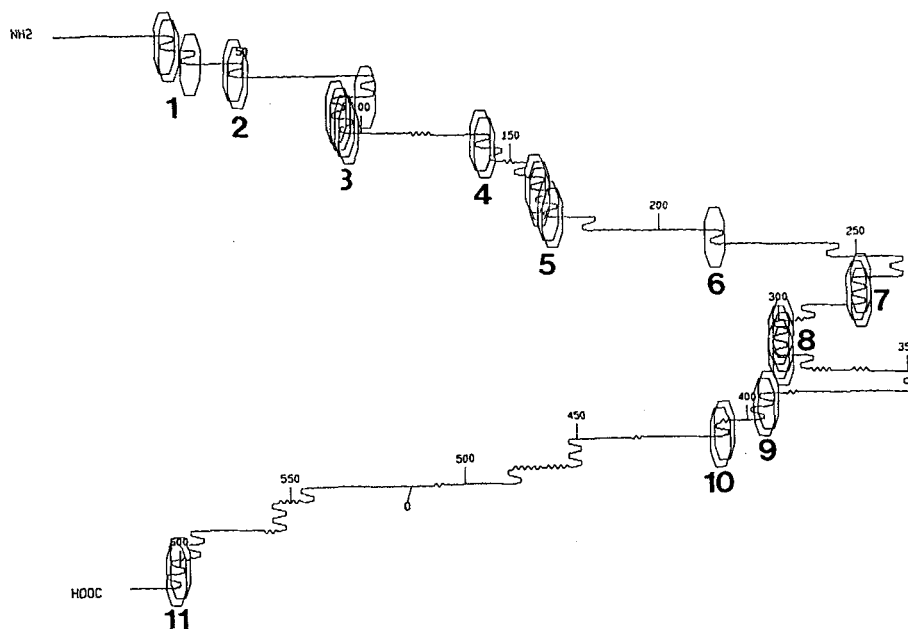


Fig. 1. There are multiple predicted antigenic sites on the Ara h 1 allergen. The amino acid sequence of the Ara h 1 protein was analyzed for potential antigenic sites by the Jameson and Wolf (1988) algorithm. There were 11 (1–11) predicted regions that contained multiple antigenic sites (octagons) along the entire length of the molecule. The amino acid sequence is represented as α -helical (sinusoidal curve), β -sheet (saw tooth curve), and coil (flat sinusoidal curve) conformations. β turns are denoted by chain reversals.

noblot analysis experiments to determine the IgE-binding characteristics of the population. At least 5 ml venous blood was drawn from each patient and allowed to clot, and the serum collected. All studies were approved by the Human Use Advisory Committee at the University of Arkansas for Medical Sciences.

Computer analysis of Ara h 1 sequence. Analysis of the Ara h 1 gene [5] and peptide sequences was performed on the University of Arkansas for Medical Sciences' Vax computer using the Wisconsin DNA analysis software package. The predicted antigenic regions on the Ara h 1 protein are based on algorithms developed by Jameson and Wolf [6] that relate antigenicity to hydrophilicity, secondary structure, flexibility, and surface probability.

Peptide synthesis. Individual peptides were synthesized on a cellulose membrane containing free hydroxyl groups using Fmoc-amino acids according to the manufacturer's instructions (Genosys Biotechnologies). Synthesis of each peptide was started by esterification of an Fmoc-amino acid to the cellulose membrane. After washing, all residual amino functions on the sheet were blocked by acetylation to render them unreactive during the subsequent steps. Fmoc protective groups were then removed by addition of piperidine to render nascent peptides reactive. Each additional Fmoc-amino acid is esterified to the previous one by this same process. After addition of the last amino acid in the peptide, the amino acid side chains were de-protected using a mixture of 1:1:0.05 (by vol.) dichloromethane/trifluoroacetic acid/triisobutylsilane, followed by washing with dichloromethane and methanol. Membranes containing synthesized peptides were either probed immediately with serum IgE or stored at -20°C until needed.

IgE-binding assay. Cellulose membranes containing synthesized peptides were incubated with the serum pool or individual serum from patients with peanut hypersensitivity diluted (1:5) in a solution containing Tris/NaCl (10 mM Tris/HCl, 500 mM NaCl, pH 7.5) and 1% bovine serum albumin for at least 12 h at 4°C or 2 h at room temperature. The primary antibody was

detected with ^{125}I -labeled anti-IgE antibody (Sanofi Pasteur Diagnostics).

RESULTS

There are multiple IgE-binding regions throughout the Ara h 1 protein. The Ara h 1 amino acid sequence was analyzed for potential antigenic epitopes using computer-based algorithms. There were 11 possible antigenic regions, each containing multiple antigenic sites, predicted by this analysis along the entire length of the molecule (Fig. 1).

77 overlapping peptides representing the entire length of the Ara h 1 protein were synthesized to determine if the predicted antigenic regions, or any other regions, were recognized by serum IgE. Each peptide was 15 amino acids long and offset from the previous peptide by eight amino acids. In this manner, the entire length of the Ara h 1 protein could be studied in large overlapping fragments. These peptides were then probed with a pool of serum IgE from 15 patients with documented peanut hypersensitivity or with serum IgE from a representative control patient with no food allergy. Serum IgE from the control patients did not recognize any of the synthesized peptides. In contrast, there are 12 IgE-binding regions (D1–D12) along the entire length of the Ara h 1 protein recognized by IgE from this population of patients with peanut hypersensitivity (Fig. 2). These IgE-binding regions represent amino acid residues 35–72, 89–112, 121–176, 289–326, 337–350, 361–374, 393–416, 457–471, 489–513, 521–535, 544–583, and 593–607. In general, the predicted antigenic regions (Fig. 2, boxed areas P1–P11) contained or were part of those that were determined (Fig. 2, shaded areas D1–D12) by actual IgE binding. However, there were two predicted antigenic regions (amino acids A221–230; amino acids 263–278) that were not recognized by serum IgE from peanut hypersensitive individuals. In addition, there were numerous IgE-binding regions found in the Ara h 1 protein between amino acids 450–600 (Fig. 2).

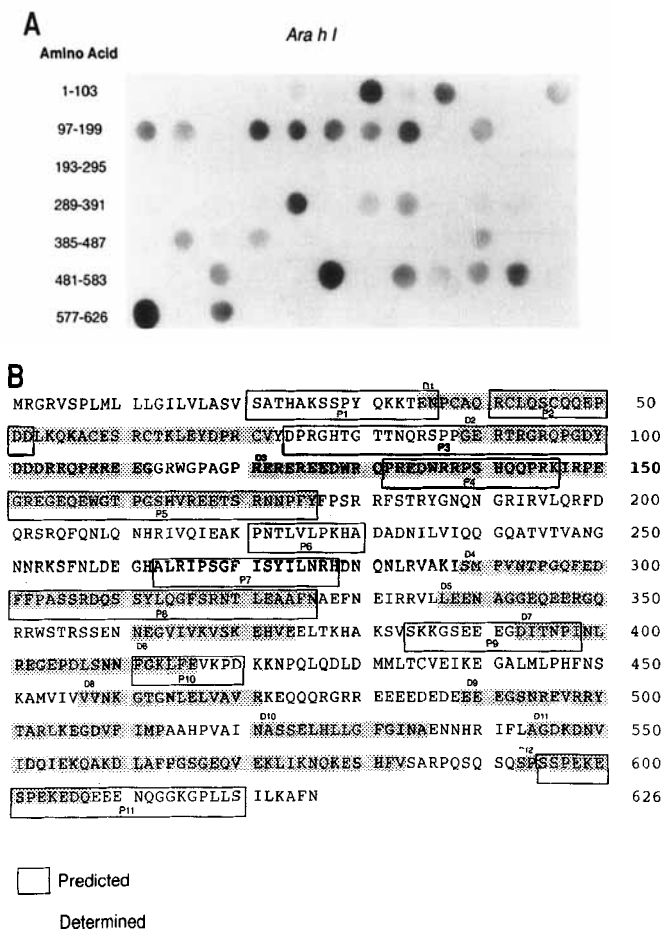


Fig. 2. Multiple IgE-binding regions identified on the Ara h 1 allergen. (A) Epitope mapping was performed on the Ara h 1 allergen by synthesizing the entire protein in 15-amino-acid-long overlapping peptides that were offset from each other by eight amino acids. These peptides were then probed with a pool of serum IgE from 15 patients with documented peanut hypersensitivity. The position of the peptides within the Ara h 1 protein are indicated on the left-hand side of A. (B) The amino acid sequence of the Ara h 1 protein is shown. The numbered boxes correspond to the predicted antigenic regions (P1–P11). The hatched areas (D1–D12) correspond to the IgE-binding regions shown in the upper panel.

To determine the amino acid sequence of the IgE-binding sites, small overlapping peptides spanning each of the larger IgE-binding regions identified in Fig. 2 were synthesized. By synthesizing smaller peptides (10 amino acids long) that were offset from each other by only two amino acids, it was possible to identify individual IgE-binding epitopes within the larger IgE-binding regions of the Ara h 1 molecule. A representative immunoblot and the respective amino acid sequence of the binding region D2–D3 (amino acids 82–133) is shown in Fig. 3. Four epitopes (Fig. 3, 4–7) were identified in this region. Similar blots were completed for the remaining IgE-binding regions to identify the core amino acid sequences for each IgE epitope. Table 1 summarizes the 23 IgE-binding epitopes (peptides 1–23) and their respective positions in the Ara h 1 molecule. The most common amino acids found were acidic (D, E) and basic (K, R) residues comprising 40% of all amino acids found in the epitopes. There were no obvious amino acid sequence motifs shared by all the IgE-binding epitopes.

Identification of common Ara h 1 epitopes recognized by serum IgE from patients with peanut hypersensitivity. In an

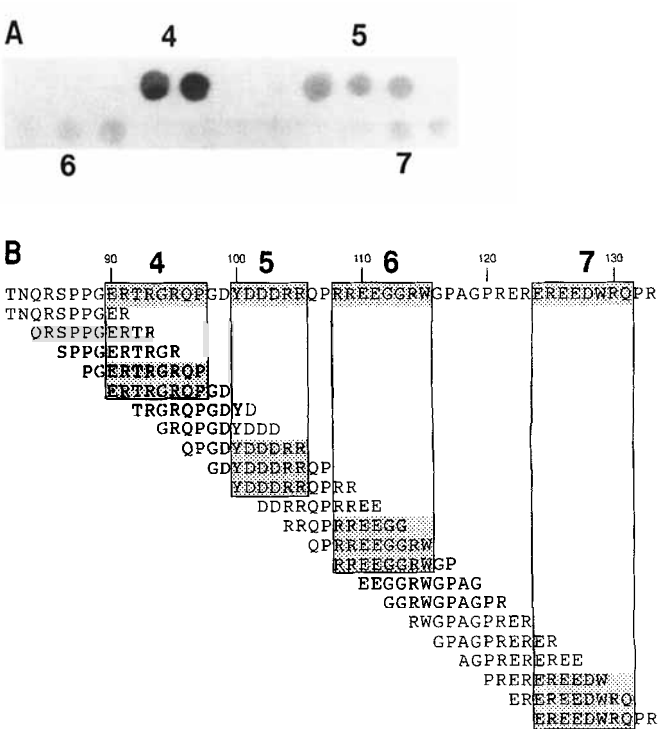


Fig. 3. Core IgE-binding epitopes identified on the Ara h 1 allergen. (A) Detailed epitope mapping was performed on IgE-binding regions identified in Fig. 2 by synthesizing ten-amino-acid-long peptides offset from each other by two amino acids. These peptides were then probed with a pool of serum IgE from 15 patients with documented peanut hypersensitivity. The data shown represents regions D2 and a portion of D3 encompassing amino acid residues 82–133. Numbers correspond to peptides as shown in Table 1. (B) The amino acid sequence (residues 82–133) of Ara h 1 that was tested in A is shown. Shaded areas of boxes correspond to common IgE-binding amino acids of the spots shown in A.

Table 1. Ara h 1 IgE-binding epitopes. The underlined portions of each peptide are the smallest IgE-binding sequences as determined by the analysis described in Fig. 3.

Peptide	Amino acid sequence	Ara h 1 positions
1	AKSSPYQKKT	25– 34
2	<u>QEPDDLKQKA</u>	48– 57
3	<u>LEYDPRLVYD</u>	65– 74
4	<u>GERTRGRQPG</u>	89– 98
5	<u>PGDYDDDRRQ</u>	97–105
6	<u>PRREEGGRWG</u>	107–116
7	<u>REEREEDWRQP</u>	123–132
8	<u>EDWRRPSHQQ</u>	134–143
9	<u>QPRKIRPEGR</u>	143–152
10	<u>TPGQFEDFFP</u>	294–303
11	<u>SYLQEFNRNT</u>	311–320
12	<u>FNAEFNEIRR</u>	325–334
13	<u>EQEERGQRRW</u>	344–353
14	<u>DITNPINLRE</u>	393–402
15	<u>NNFGKLFEVK</u>	409–418
16	<u>GTGNLELVAV</u>	461–470
17	<u>RRYTARLKEG</u>	498–507
18	<u>ELHLLGFGIN</u>	525–534
19	<u>HRIFLAGDKD</u>	539–548
20	<u>IDQIEKQAKD</u>	551–560
21	<u>KDLAFPGSGE</u>	559–568
22	<u>KESHFVSARP</u>	578–587
23	<u>PEKESPEKED</u>	597–606

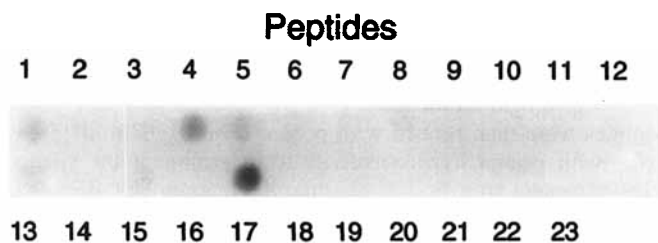


Fig. 4. Ara h 1 epitopes recognized by serum IgE from a patient with peanut hypersensitivity. IgE-binding peptides (1–23) were synthesized as ten-amino-acid peptides and probed with serum IgE from a patient with documented peanut hypersensitivity.

effort to determine which, if any, of the 23 epitopes was immunodominant, each set of 23 peptides was probed individually with serum IgE from ten different patients. An epitope can be considered immunodominant if it is recognized by serum IgE from the majority of patients with peanut hypersensitivity or if the serum IgE that recognizes a peptide represents the majority of Ara h 1-specific IgE found in a patient. Serum from five individuals randomly selected from the 15 patient serum pool and an additional five sera from peanut-hypersensitive patients not represented in the serum pool were used to identify the commonly recognized epitopes. An immunoblot strip containing these peptides that has been incubated with an individual patient's serum is shown in Fig. 4. This patient's serum IgE recog-

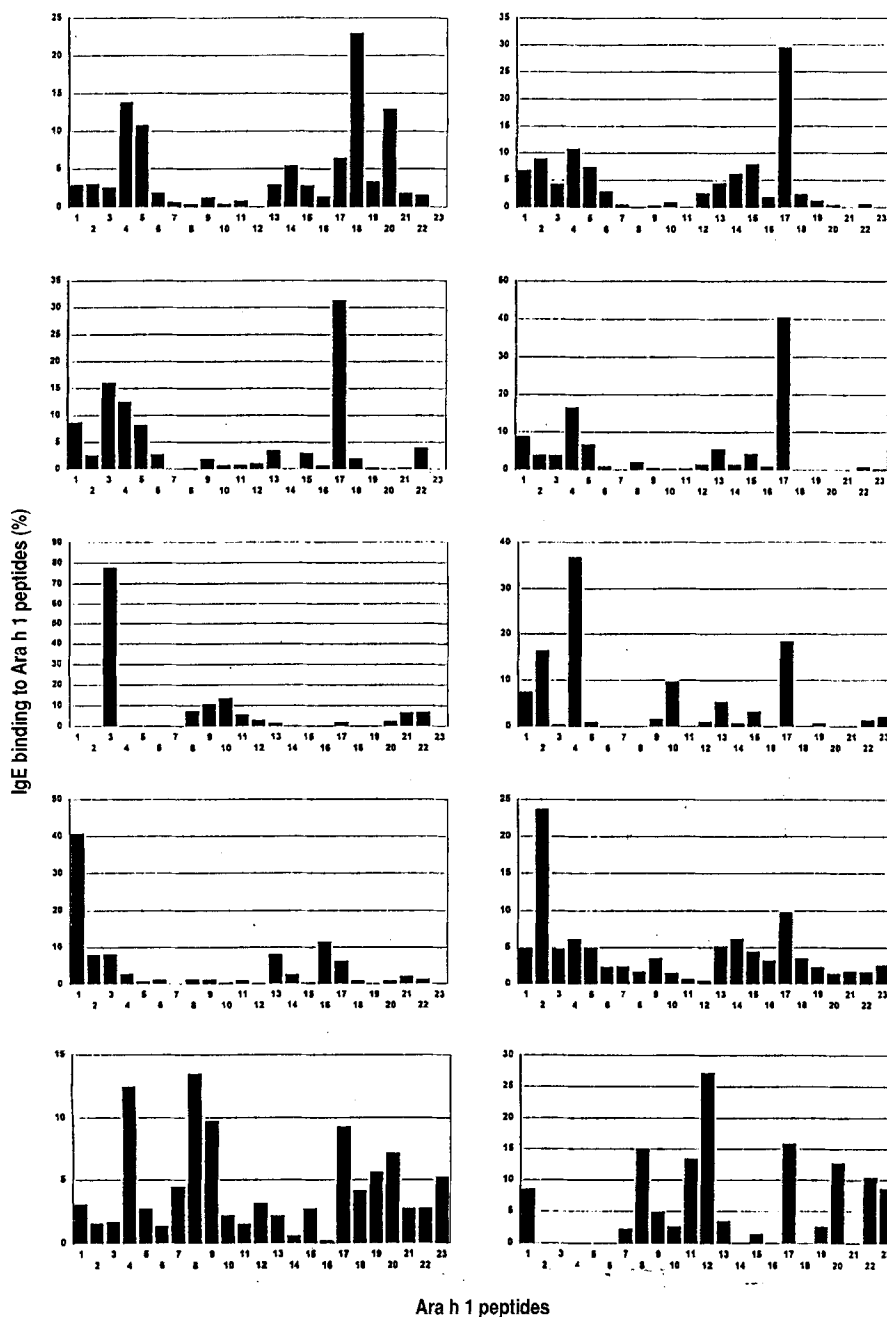


Fig. 5. Identification of the immunodominant Ara h 1 epitopes. IgE-binding peptides (1–23) were synthesized as described in Fig. 4 and probed with serum IgE from 10 patients (A–J) with peanut hypersensitivity. Serum from five individuals randomly selected from the 15-patient serum pool and an additional five sera from peanut-hypersensitive patients not represented in the serum pool were used to identify the immunodominant epitopes. The relative intensity of IgE binding to each peptide was then determined by densitometry and expressed as a percentage of the patient's total IgE binding to all of the Ara h 1 peptides.

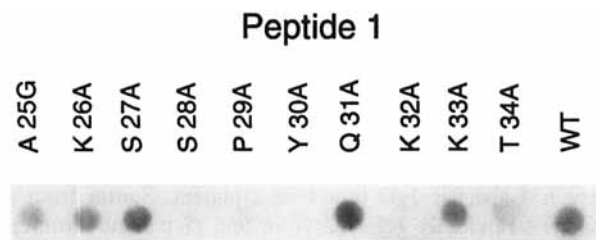


Fig. 6. Single amino acid changes to peptide 17 result in loss of IgE binding to this epitope. Peptide 17 was synthesized with an alanine residue substituted for one of the amino acids in this peptide and probed with a pool of serum IgE from 15 patients with documented peanut hypersensitivity. The letters across the top of each panel indicate the one-letter amino acid code for the residue normally at that position and the amino acid that was substituted for this residue. The numbers indicate the position of each residue in the Ara h 1 protein. WT, indicates the wild-type peptide (no amino acid substitutions).

nized peptides 1, 4, 5, 8, 13, 15, and 17. Peptides 2, 3, 6, and 9 were detected but could only be minor epitopes considering the amount of IgE that they bound. The remaining patients' serum IgE were tested in the same manner and the intensity of IgE binding to each spot was determined as a function of that patient's total IgE binding to these 23 epitopes (Fig. 5). All of the patient sera tested (10/10) recognized multiple peptides. The most commonly recognized peptides were those that contained epitopes 1, 3, 4, 13, 17, and 22. These epitopes were recognized by IgE from at least 80% of the patient sera tested (8/10). In addition, epitopes 1–4, 8, 12, and 17, when recognized, bound more serum IgE from individual patients than any of the other epitopes. These results indicate that peptides 1, 3, 4, and 17 contain the immunodominant epitopes of the Ara h 1 protein.

IgE-binding characteristics of the immunodominant Ara h 1 epitopes. The amino acids essential to IgE binding in epitopes 1, 3, 4, and 17 were determined by synthesizing duplicate peptides with single amino acid changes at each position. These peptides were then probed with pooled serum IgE from 15 patients with peanut hypersensitivity to determine if the change affected peanut-specific IgE binding. An immunoblot strip containing the wild-type and mutated peptides of epitope 1 is shown in Fig. 6. The pooled serum IgE did not recognize this peptide, or binding was drastically reduced, when alanine was substituted for each of the amino acids at positions 28–30, or 32. In contrast, the substitution of an alanine for glutamine residue at position 31 resulted in increased IgE binding. The remaining immunodominant Ara h 1 epitopes were tested in the same manner and the intensity of IgE binding to each spot was determined as a percentage of IgE binding to the wild-type peptide (Fig. 7). In general, each epitope could be mutated to a non-IgE-binding peptide by the substitution of an alanine for a single amino acid residue. There was no obvious position within each peptide that, when mutated, would result in loss of IgE binding. Furthermore, there was no consensus in the type of amino acid that, when changed to alanine, would lead to loss of IgE binding.

DISCUSSION

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 which leads to isotype switching and the generation of antigen-specific IgE. The antigen-specific IgE then binds to the FcεRI receptors of mast cells and basophils. This establishes a condition in which the IgE-bound mast cells can trigger a potentially life threaten-

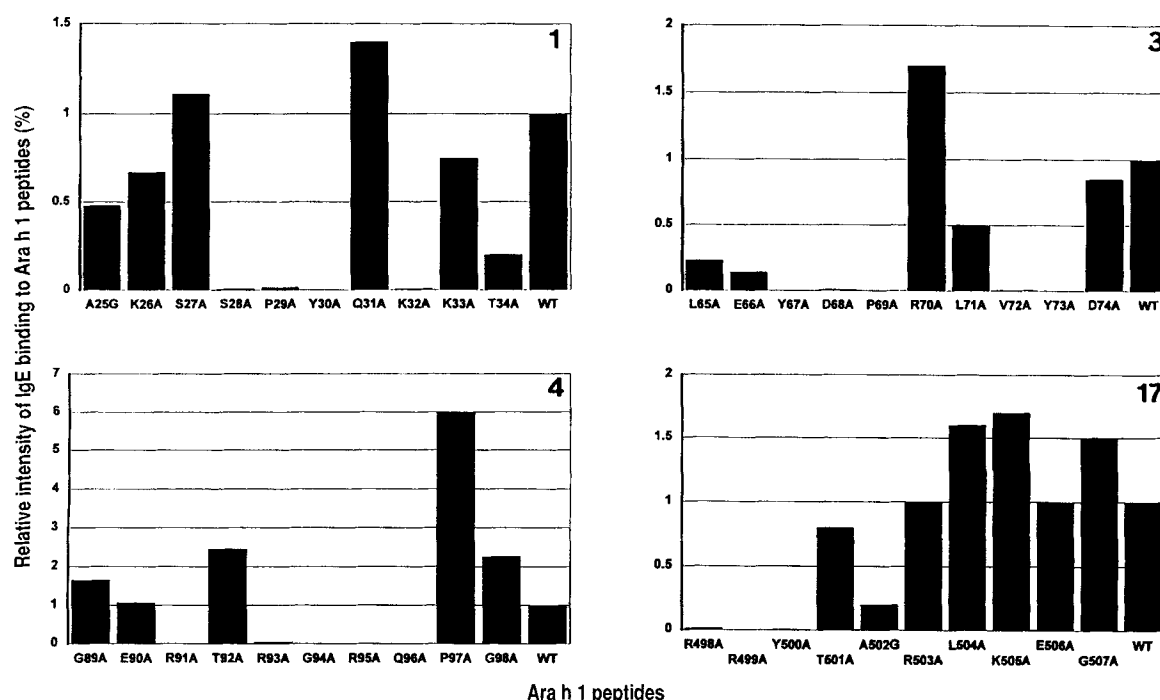


Fig. 7. All of the immunodominant Ara h 1 peptides can be mutated to non-IgE binding epitopes by single amino acid changes. All of the immunodominant Ara h 1 epitopes (peptides 1, 3, 4, and 17) were synthesized with an alanine or glycine residue substituted for one of the amino acids and probed with a pool of serum IgE from 15 patients with documented peanut hypersensitivity. The relative intensity of IgE binding to each peptide was then determined by densitometry and expressed as a percentage of IgE binding to the non-mutated peptide (WT). The letters across the bottom of each panel indicate the one-letter amino acid code for the residue normally at that position and the amino acid that was substituted for this residue. The numbers indicate the position of each residue in the Ara h 1 protein.

ing immediate hypersensitive reaction when next exposed to the allergen. Because antigen-specific IgE plays such a critical role in the etiology of allergic disease, determination of allergen-specific, IgE-binding epitopes is an important first step toward a better understanding of this complex disease process.

In the present study, we have determined that there were multiple antigenic sites predicted for the Ara h 1 allergen. In general, as has been found with other allergens [7, 8], there was good agreement between those residues predicted by computer analysis and B-cell epitopes determined by experimental analysis of overlapping peptides. This correlation between predicted and determined epitopes is probably due to the ability of the computer model to predict which regions of the molecule are exposed on the surface of the allergen, making them accessible to immunoglobulin interactions. There are at least 23 different IgE recognition sites on the major peanut allergen Ara h 1. These sites are distributed throughout the protein. The identification of multiple epitopes on a single allergen is not novel. Allergens from cow milk [9], codfish [10], hazel [11], soy [12], and shrimp [13] have all been shown to contain multiple IgE-binding epitopes. The observation that most of these proteins have multiple IgE-binding sites probably reflects the polyclonal nature of the immune response to them and may be a necessary step in establishing a protein as an allergen.

The elucidation of the major IgE-binding epitopes on Ara h 1 may also enable us to better understand the immunopathogenic mechanisms involved in peanut hypersensitivity. Recent evidence suggests that there is a preferential variable heavy-chain usage in IgE synthesis and a direct switching from IgM production to IgE synthesis [14]. This would suggest that epitopes responsible for antigen-specific IgE antibody production may differ from those promoting antigen-specific IgG antibodies. Immunotherapeutic approaches utilizing peptides representing IgG epitopes may be able to shift the balance of antigen-specific antibody production from IgE to IgG. We are currently identifying which of the IgE-binding epitopes also bind IgG to determine if this would be a feasible strategy for patients with peanut hypersensitivity.

Four of the Ara h 1 peptides appear to be immunodominant IgE-binding epitopes in that they are recognized by more than 80% of patient sera tested. Interestingly, epitope 17, which is located in the C-terminal end of the protein (amino acids 498–507), is in a region that shares significant sequence similarity with vicilins from other legumes [15]. The amino acids important for IgE binding also appear to be conserved in this region and may explain the possible cross-reacting antibodies to other legumes that can be found in sera of patients with a positive double-blind placebo-controlled food challenge to peanuts. Epitopes 1, 3, and 4 located in the N-terminal portion (amino acids 25–34, 65–74, and 89–98) of the protein, appear to be unique to this peanut vicilin and do not share any significant sequence similarity with vicilins from other legumes [15]. In addition, the amino acids important to IgE binding in this region are not conserved. These findings may enable us to develop more sensitive and specific diagnostic tools and lead to the design of novel therapeutic agents to modify the allergic response to peanuts.

Our data show that it may be possible to mutate the Ara h 1 allergen to a protein that no longer binds IgE. This raises the possibility that an altered Ara h 1 gene could be used to replace its allergenic homologue in the peanut genome. In this manner, a hypoallergenic peanut could be developed that may blunt allergic reactions in sensitive individuals who inadvertently ingest this food. Furthermore, a hypoallergenic peanut may prevent the development of peanut hypersensitivity in individuals geneti-

cally predisposed to this food allergy. Because the Ara h 1 gene product is such an abundant and integral seed storage protein [15], a prudent approach would require that the altered vicilin retain as much of its native function and properties as possible. Given the potential severity of peanut allergic reactions and the widespread use of peanuts in consumer foods, this possibility is actively being pursued in our laboratories.

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REFERENCES

1. Jansen, J. J., Kardinaal, A. F. M., Huijber, G., Vlieg-Boerstra, B. J., Martens, B. P. & Ockhuizen, T. (1994) Prevalence of food allergy and intolerance in the adult Dutch population, *J. Allergy Clin. Immunol.* **93**, 446–456.
2. Sampson, H. A. (1988) The role of food allergy and mediator release in atopic dermatitis, *J. Allergy Clin. Immunol.* **81**, 635–645.
3. Taylor, S. L., Busse, W. W., Sachs, M. I., Parker, J. L. & Yunginger, J. W. (1981) Peanut oil is not allergenic to peanut sensitive individuals, *J. Allergy Clin. Immunol.* **68**, 372–375.
4. Burks, A. W., Williams, L. W., Helm, R. M., Connaughton, C., Cockrell, G. & O'Brien, T. (1991) Identification of a major peanut allergen Ara h 1, in patients with atopic dermatitis and positive peanut challenge, *J. Allergy Clin. Immunol.* **88**, 172–179.
5. Burks, A. W., Cockrell, G., Stanley, J. S., Helm, R. M. & Bannon, G. A. (1995) Recombinant peanut allergen Ara h 1 expression and IgE binding in patients with peanut hypersensitivity, *J. Clin. Invest.* **96**, 1715–1721.
6. Jameson, B. A. & Wolf, J. (1988) The antigenic index: a novel algorithm for predicting antigenic determinants, *Comput. Appl. Biosci.* **4**, 181–186.
7. van Kampen, V., Becker, W. M., Chen, Z., Rihs, H. P., Mazur, G., Raulf, M., Liebers, V., Isringhausen-Bley, S. & Baur, X. (1994) Analysis of B-cell epitopes in the N-terminal region of Chi t 1 component III using monoclonal antibodies, *Mol. Immunol.* **31**, 1133–1140.
8. Breiteneder, H., Ferreira, F., Reikerstorfer, A., Duchene, M., Valenta, R., Hoffman-Sommergruber, K., Ebner, C., Breitenbach, M., Kraft, D. & Scheiner, O. (1992) Complementary DNA cloning and expression in *Escherichia coli* of Aln g I, the major allergen in pollen of alder (*Alnus glutinosa*), *J. Allergy Clin. Immunol.* **90**, 909–917.
9. Ball, G., Shelton, M. J., Walsh, B. J., Hill, J., Hosking, C. S. & Howden, M. E. (1994) A major continuous allergenic epitope of bovine β -lactoglobulin recognized by human IgE binding, *Clin. Exp. Allergy* **24**, 758–764.
10. Aas, K. & Elsayed, S. (1975) Physico-chemical properties and specific activity of a purified allergen (codfish), *Dev. Biol. Stand.* **29**, 90–98.
11. Elsayed, S., Holen, E. & Dybendal, T. (1989) Synthetic allergenic epitopes from the amino-terminal regions of the major allergens of hazel and birch pollen, *Int. Arch. Allergy Appl. Immunol.* **89**, 410–415.
12. Herian, A. M., Taylor, S. L. & Bush, R. K. (1990) Identification of soybean allergens by immunoblotting with sera from soy-allergic adults, *Int. Arch. Allergy Appl. Immunol.* **92**, 193–198.
13. Shanti, K. N., Martin, B. M., Nagpal, S., Metcalf, D. D. & Rao, P. V. (1993) Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes, *J. Immunol.* **151**, 5354–5363.
14. van der Stoep, N., Korver, W. & Logtenberg, T. (1994) *In vivo* and *in vitro* IgE isotype switching in human B lymphocytes: evidence for a predominantly direct IgM to IgE class switch program, *Eur. J. Immunol.* **24**, 1307–1311.
15. Gibbs, P. E., Strongin, K. B. & McPherson, A. (1989) Evolution of legume seed storage proteins – a domain common to legumins and vicilins is duplicated in vicilins, *Mol. Biol. Evol.* **6**, 614–623.