

Biochemistry of Food Allergens

J. Steven Stanley and Gary A. Bannon*

*Department of Pediatrics and Biochemistry and Molecular Biology,
University of Arkansas for Medical Sciences, Little Rock, AR 72205*

The consumer marketplace reflects widespread interest and concern about adverse reactions to certain foods and food additives. A recent consumer survey indicated 30% of the people interviewed reported that they or some family member had an allergy to a food product (1). This survey also found that 22% avoided particular foods on the mere possibility that the food may contain an allergen. The most common food allergies known to affect children are IgE-mediated reactions to cow's milk, eggs, peanuts, soybeans, wheat, fish, and tree nuts. Approximately 80% of all reported food allergies in children are owing to peanuts, milk, or eggs. Although most childhood food allergies are outgrown, allergies to peanuts, tree nuts, and fish are rarely resolved in adulthood. In adults, the most common food allergies are to peanuts, tree nuts, fish, and shellfish.

General Characteristics of Food Allergens

Identification and purification of allergens have been essential for the structural and immunologic studies necessary to understand how these molecules stimulate IgE antibody formation (2). In the past several years, a number of allergens have been identified that stimulate IgE production and cause IgE-mediated disease in humans. Significant information now exists on the identification and purification of allergens from a wide variety of sources, including foods, pollens, dust mites, animal danders, insects, and fungi (2). Most allergens are low-mol-wt proteins or glycoproteins readily soluble in aqueous solution. These properties seemingly allow rapid penetration of the allergen at mucosal membranes facilitating the immediate symptoms observed in allergic patients. Despite increasing knowledge of the structure and

*Author to whom all correspondence and reprint requests should be addressed.

amino acid sequences of the identified allergens, specific features associated with IgE antibody formation have not been determined (2).

Food allergens, in general, have the properties of any allergen that will stimulate IgE production. In comparison to the inhaled allergens, only a few food allergens known to cause IgE-mediated reactions have been similarly identified and purified (2–4). The food allergens are typically low-mol-wt glycoproteins (<70 kDa) with acidic isoelectric points that are highly abundant in the food source. These molecules are usually resistant to proteases, heat, and denaturants, allowing these food materials to resist degradation during food preparation and digestion (3).

Peanut Allergens

The peanut (*Arachis hypogaea* L.) is an annual plant belonging to the family Leguminosae (legumes) and is native to South America. Several peanut varieties are grown in the United States, including Virginia, Spanish, and runners. Various studies over the last several years have examined the nature and location of the multiple allergens in peanuts (5,6). These studies showed the allergenic portion of the peanut was in the protein of the cotyledon and does not differ significantly between the different varieties of peanuts (7).

Recent work from our laboratory and others has identified several major allergens in peanuts. The first peanut allergen identified has been named Ara h 1 (8). Ara h 1 has a mean mol wt of 63.5 kDa and an isoelectric point of 4.55. The gene for this allergen has now been cloned and sequenced, and found to have significant sequence homology with the plant vicilins (9). The second allergen isolated and purified was Ara h 2 (10). Ara h 2 has a mean mol wt of 17.5 kDa and a *pI* of 5.2 with a carbohydrate content of 20%. Additionally, the gene for this allergen has been cloned and sequenced, and found to have significant sequence homology with the plant conglutins. Greater than 95% of our patients with positive challenges to peanut had specific IgE to Ara h 1 and Ara h 2. A third peanut allergen (Ara h 3) was identified by using soy-adsorbed serum IgE from peanut-allergic patients (11). In this manner, clinically relevant allergens could be identified. A cDNA clone encoding Ara h 3 has been isolated and characterized. The deduced amino acid sequence of Ara h 3 shows high homology to the glycinin family of seed-storage proteins. The recombinant form of this allergen was expressed in a bacterial system and was recognized by serum IgE from ~50% of our peanut-allergic patient population (12).

The sequence information identified in our laboratory has shown that the gene for Ara h 1 is a part of a multigene family and that the

allergen shares significant sequence homology with the vicilin storage proteins of other legumes (e.g., soybean, pea, common bean). The major seed-storage proteins of legumes are globulins that are represented in most legumes by two different types of polypeptides, the nonglycosylated legumins and the glycosylated vicilins. All of the vicilin genes share a common ancestry. The major difference in the larger, glycosylated vicilin proteins is in the amino-terminal sequence. Similar information concerning the sequence homology exists for the conglutin and glycinin families of seed-storage proteins. Since the seed-storage proteins have significant sequence homology among many plants, serum-specific IgE would bind to several vicilin, conglutin, and glycinin proteins from different species of legumes. The information generated in our laboratory demonstrating that the major peanut allergens are seed storage proteins may explain why patients tend to have serum IgE to multiple legume proteins. Our initial studies have provided evidence that since the major differences in allergens in the multiple legumes are located in the nonhomologous regions of the storage proteins, IgE binding or the T-cell response to this portion of the proteins may be responsible for the unique, severe, and chronic hypersensitivity response to peanuts.

Serum IgE from patients with documented peanut hypersensitivity and overlapping peptides were used to identify the IgE binding epitopes on the major peanut allergens, Ara h 1, Ara h 2, and Ara h 3. At least 23 different linear IgE binding epitopes located throughout the length of the Ara h 1 molecule were identified (13). In a similar fashion, 10 IgE binding epitopes and 4 IgE binding epitopes were identified in Ara h 2 and Ara h 3, respectively (12,14). All of the epitopes were 6–15 amino acids in length, but there was no obvious sequence motif shared by all peptides. Four of the Ara h 1 epitopes appeared to be immunodominant IgE binding peptides 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 IgE binding epitopes. Similarly, three of the Ara h 2 IgE binding epitopes and one of the Ara h 3 epitopes were determined to be immunodominant.

Mutational analysis of each of the IgE binding epitopes from the peanut allergens revealed that single amino acid changes within these peptides had dramatic effects on IgE binding characteristics. Surprisingly, substitution of a single amino acid led to loss of IgE binding (13–15). Analysis of the type and position of amino acids within the IgE binding epitopes that had this effect indicated that substitution of hydrophobic residues in the center of the epitopes was more likely to lead to loss of IgE binding (15). These results represent the first systematic analysis of the IgE binding epitopes of food allergens and, as such, will be important to be taken into

consideration when developing strategies for the treatment and prevention of peanut allergy.

Egg Allergens

In the United States pediatric population, allergy to chicken egg is one of the most common food allergies (16–18). In a study of 470 children evaluated for possible food allergies by double-blind, placebo-controlled food challenges, 80% reacted to one or more foods (19). Egg proteins were responsible for as many as two-thirds of the positive double-blind, placebo-controlled food challenges. Ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), and lysozyme (Gal d 4) are the most important allergens in egg white protein (20–22). All four allergens are glycoproteins and have been cloned and sequenced. Ovomucoid makes up approx 10% of the total egg white protein and is the major allergen (23).

Ovomucoid is 186 amino acids in length, has a mol wt of 28 kDa, an isoelectric point of 4.1, and has significant homology to pancreatic secretory trypsin inhibitor. The protein is arranged into three domains of about 60 amino acid. The tertiary structure of each domain is secured by two interdomain disulfide bridges. The first two domains contain two glycosylation sites each, and the third domain contains a single site. Carbohydrate represents about 25% of the total glycoprotein's mass (24).

Recognition of ovomucoid by IgE and IgG antibody isotypes is resistant to extensive heating, proteolytic digestion, deglycosylation, and chemical denaturation (25–27). These observations suggest that linear epitopes are important in antibody recognition of ovomucoid. In a study designed to evaluate the importance of linear and conformation epitopes for both IgE and IgG antibodies, a great deal of person-to-person variability was found. It was noted that as a general rule in newly acquired egg hypersensitivity, antibody binding was more dependent on conformational epitopes, whereas linear epitopes were more important in individuals who had had their allergies longer. The linear IgE and IgG binding epitopes of ovomucoid have been mapped using overlapping synthetic dodecapeptides representing the full-length protein. Pooled serum IgE from seven egg-allergic individuals recognized five regions of the ovomucoid protein, but seven regions were recognized by the pooled IgG. Most of the IgE antibody binding sites also bound IgG.

The allergen ovalbumin (Gal d 2) is a 42.8-kDa glycoprotein that represents more than 50% of the protein in egg white. The importance of linear IgE epitopes was determined when it was observed that much of the IgE binding to ovalbumin is resistant to enzymatic or chemical

degradation and denaturation (28,29). Using chemical and enzymatic digestion of the full-length protein and synthetic peptides based on the known protein sequence, at least seven IgE binding regions have been identified by several different laboratories. The IgE epitopes identified so far cluster at the amino- and carboxyl-ends of the allergen (30).

Ovotransferrin (Gal d 3) and lysozyme (Gal d 4) have not been studied to the same degree as ovomucoid and ovalbumin. Ovotransferrin is an ~70-kDa iron binding glycoprotein with bactericidal activity in egg white and an isoelectric point between pH 5.6 and 6.2. Lysozyme is an ~14.3-kDa glycoprotein with an isoelectric point at pH 11.0 (22). The four allergens ovomucoid, ovalbumin, ovotransferrin, and lysozyme account for approx 80% of the protein in egg white.

Milk Allergens

Cow's milk is one of the first foods added to an infant's diet and tends to be one of the most allergenic foods. Serum IgE antibodies have implicated α -casein (~32.4 kDa) and β -casein (~26.6 kDa), α -lactalbumin (~14.2 kDa), β -lactoglobulin (~18 kDa), bovine serum albumin (~66 kDa), and bovine γ -globulin (~150 kDa) as milk allergens. However, α -casein (~32.4 kDa) and β -lactoglobulin (~26.6 kDa) appear to be the most common allergens for milk-allergic individuals (31).

Bovine α_{S1} -casein is a very acidic phosphoprotein with an isoelectric point of pH 4.1–4.5. The structure of α_{S1} -casein is dominated by three attributes of its 199 amino acid primary structure. Nearly half of its 199 amino acids are hydrophobic, 8 of its 16 serine residues may be phosphorylated, and there are 17 proline residues located throughout its length. These factors, plus a lack of disulfide bridges, lead to the protein exhibiting more linear conformation with reduced tertiary interactions (32). This lack of higher-order structure increases the likelihood that important IgE binding epitopes are linear and not conformational. Two groups have recently attempted mapping the linear IgE and IgG binding epitopes of α_{S1} -casein. Spuerger et al. (33) used pooled serum from 15 cow's milk-allergic individuals and overlapping synthetic peptides to identify seven IgE binding epitopes. Three of these epitopes were recognized by IgE from the sera of all 15 individuals. These three immunodominant epitopes were distinguished by a high content of nonpolar and aromatic amino acids. The IgG binding epitopes were mapped to essentially the same positions in α_{S1} -casein as the IgE binding epitopes. Nakajima-Adachi et al. (34) mapped IgE, IgG₄, and T-cell determinants of α_{S1} -casein using overlapping synthetic peptides in combination with digestion fragments. A single immunodominant IgE binding peptide was identified at the

carboxyl-terminal end of the allergen. The peptide was designated P13 and corresponds to the 20 amino acids at position 181–199 of α_{S1} -casein. P13 was recognized by serum IgE from all nine milk-allergic patients used in this study. One other peptide, P8 (amino acids 106–125), was recognized by a single individual. These results are very different from those of Spuerger et al. (33), who identified only two minor epitopes in this area (position 172–183 and 189–199) and major epitopes at positions where Nakajima-Adachi et al. (34) saw no IgE binding. The difference in results may be owing to differences in the way the peptides were made or the population of milk-allergic patients used in the two studies. Spuerger et al. (33) used 188 peptides that were 10 residues long and overlapped by 9 residues. Nakajima-Adachi et al. (34) used 13 peptides 20 residues long and overlapping by 5 residues on both the N- and C-terminus. Both groups measured IgE binding to the synthetic peptides by enzyme-linked immunosorbent assay (ELISA) and used different formulas to decide what level of binding was significant. The formula used by Nakajima-Adachi et al. (34) appeared to be more stringent and may explain why they mapped fewer IgE binding sites. In addition, it is possible that because one study was carried out in Germany and the other in Japan, there is a difference in the two allergic populations. Nakajima-Adachi et al. (34) reported no difference in the peptides bound by IgE and IgG₄ which agrees with the findings of Spuerger et al. (33).

Using T-cell lines isolated from two individuals, Nakajima-Adachi et al. (34) identified six different T-cell epitopes distributed throughout the length of α_{S1} -casein. When the T-cell epitopes recognized by each individual were compared, two different binding motifs were observed. The T-cell lines isolated from the first individual recognized peptides with a glutamic acid anchor residue at the amino terminal end and a leucine anchor residue at the carboxyl-terminal end separated by seven amino acids (-E-[X]₇-L-). The T-cell lines from the second individual recognized peptides with a glutamic acid anchor residue at the amino-terminal end and a lysine anchor residue at the carboxyl-terminal end separated by six amino acids (-E-[X]₆-K-). Interestingly, a T-cell-activating motif with the arrangement of -E-(X)₅-K- has been identified as a crossreacting T-cell epitope present in a number of different inhaled allergens. This motif is very similar to the -E-(X)₆-K- motif found by Nakajima-Adachi et al. (34). In addition, α_{S1} -casein contains the -E-(X)₅-K- at two other positions in the sequence. Although none of the seven T-cell lines from the two individuals used in this study recognized this crossreactive epitope, it might very well play a role in vivo in these individuals or in others.

Fish and Shellfish Allergens

Allergic reactions to fish and shellfish are common causes of food allergy in both children and adults. Fish and shellfish allergies resemble peanut allergy in that they are less likely to resolve naturally with age. Two allergens that have been extensively studied are Gad c 1 and Pen a 1. Gad c 1 is the parvalbumin homolog present in codfish. It is a glycoprotein approx 12.5 kDa in size. Parvalbumin is present in most fish species examined and is generally the most IgE-reactive protein present (35,36). As yet, the IgE binding epitopes of Gad c 1 have not been mapped.

Tropomyosins (~36 kDa) is a major allergen identified in shrimp. Pen a 1 is the brown shrimp (*Penaeus aztecus*) homolog of tropomyosin, and Met e 1 is the tropomyosin homolog isolated from the greasyback shrimp (*Metapenaeus ensis*). Recently, tropomyosin was implicated as an allergen in lobster (37). Pan s 1 from the spiny lobster (*Panulirus stimpsoni*) and Hom a 1 from the American lobster (*Homarus americanus*) are both tropomyosin homologs. Both allergens have been cloned and sequenced, and have significant homology to the shrimp allergen Pen a 1. Inhibition studies show that the two lobster allergens will completely inhibit each other's binding of IgE from lobster-allergic individuals. In addition, the recombinant shrimp allergen Met e 1 is able to block IgE binding to lobster. This suggests that tropomyosin is also the major allergen in lobster and that many of the IgE binding epitopes are crossreactive (37).

Tropomyosins are also present in vertebrate sources of food (e.g., bony fish, beef, pork, and chicken), yet are rarely allergenic in these foods. This has generated interest in the molecular characteristics that might explain this difference in allergenicity. Reese et al. mapped IgE-reactive peptides from Pen a 1 using a peptide expression library and serum IgE from shrimp-allergic patients (38). Four peptides (13–21 amino acids long) were identified and sequenced. All four peptides were located in the carboxyl-terminal end of the allergen. The peptides aligned with both conserved and nonconserved regions of tropomyosin. However, none of the peptides were identical with the vertebrate homologs. This observation suggests that the amino acid differences between the vertebrate and shrimp tropomyosin may be responsible for the differences in allergenicity. However, experiments involving site-directed mutagenesis to determine which of the amino acid residues are critical for IgE binding have not yet been performed.

Strategies to Alter Food Allergens

The prevalence of food allergy is believed to be between 2 and 8% for infants and children, and approx 1% for the adult population

(39,40) In addition, the incidence of all allergic diseases appears to be on the increase in industrialized societies (41). Immunotherapy to treat food allergies is an area of intense study. Inhalant allergies and insect sting allergies can be treated with traditional desensitization therapy. However, desensitization therapy for food allergies has been associated with life-threatening anaphylactic reactions and is not recommended. Elimination diets, in which the allergen is identified and then excluded from the diet, are presently the only proven therapy for food allergies. Adhering to elimination diets for foods, such as milk, egg, soy, wheat, and peanut, without accidentally ingesting the offending allergen is very difficult. In addition, eliminating important foods like milk from the diet introduces the risk of negatively affecting the nutritional state of the allergic patient. In a study of calcium intake in a population of 58 patients diagnosed as allergic to cow's milk, 53% of the patients were found to fall below the recommended daily allowance (RDA) for calcium (42). Twenty-one percent of the patients were given calcium supplements, but still did not reach their RDA. Altering the allergen to make it nonallergenic would alleviate many of the problems with elimination diets.

Physiochemical and Enzymatic Processing

Instead of treating the clinical symptoms of allergy, an alternative approach is to modify the allergenic proteins so that they no longer elicit a response. This approach has a successful history beginning more than 50 years ago with the first processes for hydrolyzing milk formulas. Food allergens are generally resistant to extremes of heat, pH, and enzymatic degradation. Resistance to denaturation and degradation allows the allergen to survive the digestive system long enough to interact with the immune system either to sensitize the individual or to trigger a reaction. Still some allergens are labile and do not survive processing. For example, many fruit allergens are heat-sensitive and are destroyed in the canning process. Enzymatic hydrolysis of food proteins, however, is the most effective and common method for reducing the danger posed by food allergens. Allergies to cow's milk are not only one of the most common, but also one of the most problematic food allergies, because there are so few alternatives for infants. Soybeans are also a common food allergen, and switching from a cow's milk-based formula to a soybean-based formula will often result in allergy to soybeans (43). Because of this, infant formulas using enzymatic hydrolysis of cow's milk are common substitutes for treating this allergy. Hydrolyzed formulas can be classified as either partially hydrolyzed or extensively hydrolyzed. Although the partially hydrolyzed cow's milk is reduced in allergenicity, it still

contains full-length allergen and large allergen fragments capable of binding IgE and triggering allergic reactions. For this reason, it is not suitable for treating cow's milk allergy (44). Extensively hydrolyzed formulas have been studied to determine the extent to which the milk has been rendered hypoallergenic. Generally, these studies have detected allergen derived peptides still capable of binding IgE and/or triggering positive prick skin tests. However, it was rare for the extensively hydrolyzed formulas to trigger allergic reactions in patient challenges (45–48). In addition to extensive enzymatic hydrolysis of the allergenic proteins, the formula is generally filtered to remove any protein larger than about 8 kDa. This filtration removes any allergens that may have escaped hydrolysis. Enzymatic hydrolysis, since it is specific for proteins and has no effect on the lipid, carbohydrate, or mineral content of the food source, does little harm to the nutritional value of the food. The peptides and amino acids that result are easily taken up and utilized by the digestive tract. Two problems with the extensively hydrolyzed cow's milk formulas are the bitter taste and an increase in osmotic pressure caused by the increased concentration of amino acids. Another very effective method of producing a hypoallergenic formula has been to base the formula entirely on free amino acids (45,48). Avoiding the use of intact proteins from the very beginning prevents the potential for allergens ending up in the finished product.

Enzymatic hydrolysis can also be used on nonmilk foods. A method was developed to reduce the allergenicity of rice using proteolytic enzymes and surfactants. The resulting hypoallergenic rice had little or no IgE binding and was well tolerated by rice-allergic patients (49). Proteolytic methods for destroying the allergens of wheat have also been described (50).

Traditional Plant Breeding Methods and Genetic Engineering

Removing the allergenic protein from the food source through breeding or genetic engineering would be an economical and desirable alternative to chemical or enzymatic hydrolysis. Celiac disease is a non-IgE-mediated food hypersensitivity to wheat gliadins. Strains of wheat lacking different gliadin genes have been bred to produce wheat lacking most of the gliadin proteins. The same strategy could be used to breed naturally hypoallergenic strain of allergenic fruits and vegetables where such strains exist. Apples are an example of an allergenic fruit in which the concentration of the allergenic protein, Mal d 1, varies greatly between different strains (51). Therefore, hypoallergenic strains of apple might be developed and substituted for the more

allergenic strains in processed foods. Peanut strains, on the other hand, all contain similar amounts of the same major allergens. The major allergens of the peanut are storage proteins laid down by the plant to supply amino acids for the growth and development of the peanut embryo. These proteins are abundant, important economically, and required for seed germination. Thus, developing hypoallergenic peanuts by traditional breeding methods will be difficult. Genetic engineering may offer a way to deal with allergens, such as those found in the peanut. We have been very successful in identifying the IgE binding epitopes of the three major peanut allergens (12–14). These epitopes have been mutated to a non-IgE binding form in the recombinant allergen using site-directed mutagenesis. Care has been taken in the mutagenesis to avoid disturbing the protein folding. This process should produce a hypoallergenic protein able to carry out the same functions as the unaltered allergen. In future studies, we hope to replace the genomic version of all three peanut allergens with the hypoallergenic mutants. The potential advantages of this approach are numerous and exciting. Allergen avoidance is currently the only effective therapy for food allergy. However, the long-term nature of the peanut hypersensitivity and the increasing use of peanuts and their byproducts as additives in other foods make avoidance very difficult. Bock and Atkins found that of 32 peanut-allergic individuals trying to eliminate peanuts from their diets, 24 had accidentally consumed peanuts within the last 5 yr, 16 within the last year alone (52). Not only will the hypoallergenic version of the peanut be safer if accidentally ingested, but it may actually reduce the number of new individuals becoming sensitized to peanuts.

A great deal of thought and research has been given to the mechanisms that determine which proteins will be allergenic and which benign. Resistance to pH, heat, and proteolytic enzymes have been suggested as possible requirements for food allergens (53). However, it is doubtful that these properties alone are sufficient to make a food protein allergenic. The possibility exists that the allergen is simply the target of the immune system, and that a second protein or some non-protein compound is acting as an adjuvant and triggering the atopic immune response. If the allergen is not responsible for triggering the atopic response itself, then substituting a non-IgE binding mutant for the original allergen may not result in lowering the number of people who become sensitized to peanuts. However, this problem could be addressed by introducing pepsin cleavage sites at exposed positions in the folded protein, thus creating a labile protein without affecting the protein's function. If resistance to pepsin is required for food allergens, then making the allergens labile to enzymatic cleavage should disrupt the sensitization process.

Summary and Prospectus

The proteins within foods that are allergenic represent a wide variety of proteins with functions that vary from storage to enzymatic activity. General characteristics of food allergens include an acidic isoelectric point, abundance in the food, stability to digestion, and the presence of multiple, linear IgE binding epitopes. However, there are exceptions even to these very general characteristics making it nearly impossible to predict whether a protein is a potential allergen. Since the development of an IgE response to an allergen involves a series of interactions among antigen-presenting cells (APCs), T-cells, and B-cells, it is important to continue to characterize both B-cell and T-cell epitopes of known allergens. With this information, it will be possible to improve diagnosis, develop new immunotherapeutic strategies, and eventually develop hypoallergenic plants for a safer food supply.

References

1. Sloan AE, Powers ME. A perspective on popular perceptions of adverse reactions to foods. *J Allergy Clin Immunol* 1986; 78:127–133.
2. Anderson JA, Sogn DD (eds.) Adverse reactions to foods. NIH Publication No. 84-2442, 1984, pp. 1–6.
3. Metcalfe DD. Food allergens. *Clin Rev Allergy* 1985; 3:331–349.
4. Lemanske RF, Taylor SL. Standardized extracts, Foods. *Clin Rev Allergy* 1987; 5:23–36.
5. Bush RK, Taylor SL, Nordlee JA. Peanut sensitivity. *Allergy Proc* 1989; 10:261–264.
6. Gillespie DN, Nakajima S, Gleich GJ. Detection of allergy to nuts by the radio-allergosorbent test. *J Allergy Clin Immunol* 1976; 57:302–309.
7. Taylor SL, Busse WW, Sachs MI, Parker JL, Yunginger JW. Peanut oil is not allergenic to peanut sensitive individuals. *J Allergy Clin Immunol* 1981; 68:372–375.
8. 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; 88:172–179.
9. 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; 96:1715–1721.
10. 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 challenge. *J Allergy Clin Immunol* 1992; 90:962–969.
11. Eigenmann 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; 98:969–978.
12. Rabjohn P, Helm E, Stanley JS, West CM, Sampson HA, Burks AW, et al. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J Clin Invest* 1999; 103:535–542.
13. Burks AW, Shin D, Cockrell G, Stanley JS, Helm R, Bannon GA. Mapping and mutational analysis of the IgE binding epitopes on Ara h I, a legume storage protein and a major peanut allergen. *Eur J Biochem* 1997; 245:334–339.

14. Stanley JS, King N, Burks AW, Huang SK, Sampson H, Cockrell G, et al. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch Biochem Biophys* 1997; 342:244-253.
15. Shin D, Compadre C, Maleki S, Kopper R, Sampson H, Huang S-K, Burks AW, 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; 273:13753-13759.
16. Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J Pediatr* 1985; 107:669.
17. Bock SA, Atkins FM. Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. *J Pediatr* 1990; 117:561.
18. Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual [see comments] [Review] [29 refs]. *J Allergy Clin Immunol* 1988; 82:986.
19. Sampson HA. Food sensitivity and the pathogenesis of atopic dermatitis [Review] [56 refs]. *J Roy Soc Med* 1997; 90 Suppl 30:2.
20. Langeland T. A clinical and immunological study of allergy to hen's egg white. III. Allergens in hen's egg white studied by crossed radio-immuno-electrophoresis (CRIE). *Allergy: Eur J Allergy Clin Immunol* 1982; 37:521.
21. Hoffman DR. Immunochemical identification of the allergens in egg white. *J Allergy Clin Immunol* 1983; 71:481.
22. Holen E, Elsayed S. Characterization of four major allergens of hen egg-white by IEF/SDS-PAGE combined with electrophoretic transfer and IgE-immunautoradiography. *Int Arch Allergy Appl Immunol* 1990; 91:136.
23. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994; 93:1047.
24. Egge H, Peter-Katalinic J, Paz-Parente J, Strecker G, Montreuil J, Fournet B. Carbohydrate structures of hen ovomucoid. A mass spectrometric analysis. *FEBS Lett* 1983; 156:357.
25. Matsuda T, Watanabe K, Nakamura R. Immunochemical studies on thermal denaturation of ovomucoid. *Biochim Biophys Acta* 1982; 707:121.
26. Honma K, Aoyagi M, Saito K, Nishimuta T, Sugimoto K, Tsunoo H, et al. Antigenic determinants on ovalbumin and ovomucoid: comparison of the specificity of IgG and IgE antibodies [Japanese]. *Arerugi—Jpn J Allergol* 1991; 40:1167.
27. Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. *J Immunol* 1997; 159:2026.
28. Honma K, Kohno Y, Saito K, Shimojo N, Tsunoo H, Niimi. Specificities of IgE, IgG and IgA antibodies to ovalbumin. Comparison of binding activities to denatured ovalbumin or ovalbumin fragments of IgE antibodies with those of IgG or IgA antibodies. *Int Arch Allergy Immunol* 1994; 103:28.
29. Kahlert H, Petersen A, Becker WM, Schlaak M. Epitope analysis of the allergen ovalbumin (Gal d II) with monoclonal antibodies and patients' IgE. *Mol Immunol* 1992; 29:1191.
30. Honma K, Kohno Y, Saito K, Shimojo N, Horiuchi T, Hayashi H, et al. Allergenic epitopes of ovalbumin (OVA) in patients with hen's egg allergy: inhibition of basophil histamine release by haptenic ovalbumin peptide. *Clin Exp Immunol* 1996; 103:446.
31. Docena GH, Fernandez R, Chirido FG, Fossati CA. Identification of casein as the major allergenic and antigenic protein of cow's milk. *Allergy: Eur J Allergy Clin Immunol* 1996; 51:412.
32. Kumosinski TF, Brown EM, Farrell HM, Jr. Three-dimensional molecular modeling of bovine caseins: alpha s1-casein. *J Dairy Sci* 1991; 74:2889.

33. Spuergerin P, Mueller H, Walter M, Schiltz E, Forster J. Allergenic epitopes of bovine alpha S1-casein recognized by human IgE and IgG. *Allergy: Eur J Allergy Clin Immunol* 1996; 51:306.
34. Nakajima-Adachi H, Hachimura S, Ise W, Honma K, Nishiwaki S, Hirota M, et al. Determinant analysis of IgE and IgG₄ antibodies and T cells specific for bovine alpha(s)1-casein from the same patients allergic to cow's milk: existence of alpha(s)1-casein-specific B cells and T cells characteristic in cow's-milk allergy. *J Allergy Clin Immunol* 1998; 101:660.
35. James JM, Helm RM, Burks AW, Lehrer SB. Comparison of pediatric and adult IgE antibody binding to fish proteins. *Ann Allergy Asthma Immunol* 1997; 79:131.
36. Hansen TK, Bindslev-Jensen C, Skov PS, Poulsen LK. Codfish allergy in adults: IgE cross-reactivity among fish species. *Ann Allergy Asthma Immunol* 1997; 78:187.
37. Leung PS, Chen YC, Mykles DL, Chow WK, Li CP, Chu KH. Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. *Mol Marine Biol Biotechnol* 1998 ;7:12.
38. Reese G, Jeoung BJ, Daul CB, Lehrer SB. Characterization of recombinant shrimp allergen Pen a 1 (tropomyosin). *Int Arch Allergy Immunol* 1997; 113:240.
39. Sampson HA Food allergy [Review] [73 refs]. *JAMA* 1997; 278:1888.
40. Sampson HA, Burks AW. Mechanisms of food allergy [Review] [83 refs]. *Ann Rev Nutr* 1996; 16:161.
41. Hanson L, Telemo E. The growing allergy problem [Review] [34 refs]. *Acta Paediatr* 1997; 86:916.
42. Madsen CD, Henderson RC. Calcium intake in children with positive IgG RAST to cow's milk. *J Paediatr Child Health* 1997; 33:209.
43. Bishop JM, Hill DJ, Hosking CS. Natural history of cow milk allergy: clinical outcome [see comments]. *J Pediatr* 1990; 116:862.
44. Halken S, Host A. How hypoallergenic are hypoallergenic cow's milk-based formulas? [Review] [53 refs]. *Allergy: Eur J Allergy Clin Immunol* 1997; 52:1175.
45. Sampson HA, James JM, Bernhisel-Broadbent J. Safety of an amino acid-derived infant formula in children allergic to cow milk. *Pediatrics* 1992; 90:463.
46. Rugo E, Wahl R, Wahn U. How allergenic are hypoallergenic infant formulae? [see comments]. *Clin Exp Allergy* 1992; 22:635.
47. Halken S, Host A, Hansen LG, Osterballe O. Safety of a new, ultrafiltrated whey hydrolysate formula in children with cow milk allergy: a clinical investigation. *Pediatr Allergy Immunol* 1993; 4:53.
48. Isolauri E, Sutas Y, Makinen-Kiljunen S, Oja SS, Isosomppi R, Turjanmaa K. Efficacy and safety of hydrolyzed cow milk and amino acid-derived formulas in infants with cow milk allergy. *J Pediatr* 1995; 127:550.
49. Watanabe M, Miyakawa J, Ikezawa Z, et al. 1990. Production of hypoallergenic rice by enzymatic decomposition of constituent proteins. *J Food Sci* 1990; 55:781.
50. Tanabe S, Arai S, Watanabe M. Modification of wheat flour with bromelain and baking hypoallergenic bread with added ingredients. *Biosci Biotechnol Biochem* 1996; 60:1269.
51. Vieths S, Jankiewicz A, Schonung B, Aulepp H. Apple allergy: the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen. *Allergy: Eur J Allergy Clin Immunol* 1994; 49:262.
52. Bock SA, Atkins FM. The natural history of peanut allergy. *J Allergy Clin Immunol* 1989; 83:900.
53. Astwood JD, Leach JN, Fuchs RL. Stability of food allergens to digestion in vitro. *Nature Biotechnol* 1996; 14:1269.