

Current Understanding of Food Allergens

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ABSTRACT: Food allergies are IgE-mediated immunological reactions; this distinguishes them from other adverse reactions to foods. Most (>90%) of the recognized food allergies are generally thought to be caused by eight foods or food groups. A number of factors can affect food allergy development, including diet and culture, route of exposure, processing, cooking, and digestion. In addition, it is thought that the properties of certain food proteins render them more likely to be allergenic than other proteins. Most food allergens are major proteins, polyvalent molecules with at least two or more IgE-binding sites, and are recognized as foreign molecules (hence immunogenic). A number of major food allergens have been recently characterized, and amino acid sequences determined. Tropomyosin is the only major allergen of shrimp. A number of IgE-binding epitopes have been identified in this molecule, though they may vary from one shrimp-allergic individual to another. Single amino acid substitutions within epitopes based on that of homologous, nonreactive tropomyosins can substantially enhance or abolish IgE antibody binding. Using the accumulated knowledge of food allergen protein structure, the allergenicity of novel proteins to which there has been no prior human exposure has been assessed. This has been based primarily on the lability or resistance of a protein to enzymatic degradation. Clearly, further criteria must be developed to refine this process. In this regard, the development of animal models that have been sufficiently validated as surrogates of human IgE antibody responses is needed for more precise assessment of the allergenic potential of proteins.

KEYWORDS: food allergens; metabolic food intolerance; gut-associated lymphoid tissue; shrimp tropomyosin

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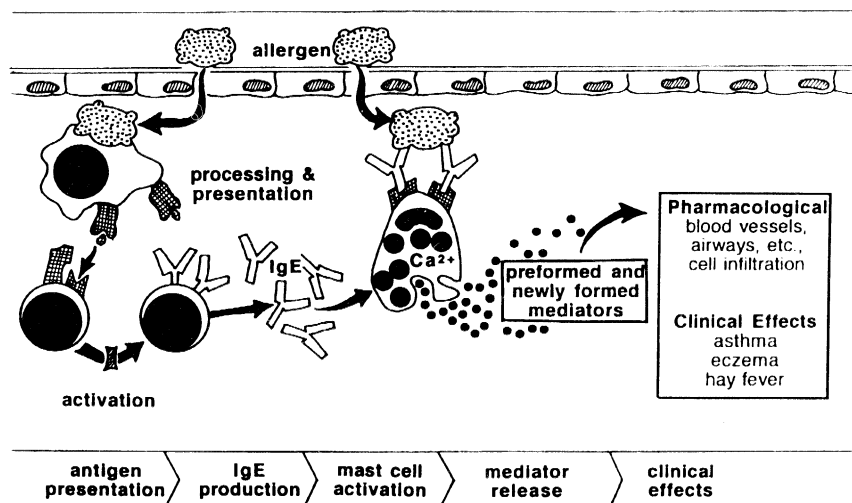


FIGURE 1. Induction and effective mechanisms in IgE-mediated hypersensitivity: An allergen that crosses mucosal membrane barriers is processed and presented to IgE-producing cells (antigen presentation). This stimulates the production of IgE antibodies. These antibodies have the ability to bind to mast cell surfaces (mast cell activation); subsequent interaction with allergen can cross-link two or more membrane-bound IgE molecules, which stimulates the release of preformed and newly synthesized mediators of allergy. These mediators result in the clinical effects of allergic reactions.

INTRODUCTION

It has been estimated that approximately 1 to 2% of the adult population and 4 to 8% of the pediatric population suffer from food allergies.¹⁻³ Most of the recognized food allergies (more than 90%) are generally thought to be caused by eight foods or food groups. These include crustacea, eggs, fish, milk, peanuts, soybeans, tree nuts, and wheat. The symptoms of food allergy vary considerably from mild discomfort, such as itching of the skin or throat, to profound, life-threatening, systemic anaphylactic shock. Food allergies are IgE-mediated immunological reactions, which distinguishes them from other adverse reactions to foods that may include metabolic food intolerances or toxic food reactions.³

The induction of allergic reactions in general, and food allergic reactions specifically, is summarized in FIGURE 1. An allergen, or an immunologically intact fragment of allergen resistant to cooking and digestive processes, crosses the epithelial barrier of the gut. After processing and presentation by

antigen-presenting cells (generally macrophages), immunologically active fragments are recognized by T lymphocytes, which along with other allergen fragments stimulate B lymphocytes to synthesize and ultimately produce IgE antibodies specific for that allergen of stimulation. IgE antibodies have the unique ability to fix to the surfaces of mast cells or basophils. Upon a second or subsequent interaction of allergen with cell-bound IgE molecules, events are initiated in the membrane of these cells leading to the release of both pre-formed and newly formed mediators. These mediators are molecules that have potent pharmacological reactions resulting in the clinical effects that are seen in allergic reactions.^{1,2} When one views the events leading to the induction and manifestation of allergic reaction, there are clearly certain features imposed on these allergenic molecules by their biological activity. For example, food allergens must be rather hearty molecules to resist the processes of cooking and digestion; they must be small enough to cross the epithelial barrier of the gut, yet of sufficient size to remain in an immunologically intact form. Finally, these molecules must be at least divalent and most probably polyvalent to cross-link cell-bound IgE molecules resulting in allergic reactions.⁴⁻⁶

There are a number of factors that can affect food allergy development. Diet and culture can have a significant effect on food allergies that manifest themselves in a particular region. For example, codfish allergy is most prevalent in Norway because codfish is an important source of protein for Norwegians. Similarly, rice and soy allergy are more prevalent in Japan, and peanut allergy is more prevalent in the United States. The route of exposure can also have an important effect on food-induced allergic reactions. Clearly, ingestion is a much harsher route of allergen exposure and thus may require higher doses in order for a sufficient number of molecules or allergenically active molecular fragments to survive in an immunologically intact form. However, individuals can also be sensitized through inhalation of allergen, a less harsh route in which lower doses of allergen can stimulate a response.⁷ This occurs with occupational sensitization of food workers.⁸ The relationship of inhalation to ingestion exposures of food allergen or the cross-reactivity of food allergens with inhaled allergens in the pathogenesis of food allergy is only now beginning to be understood.

Processing, cooking, and digestion obviously can have a profound effect on foods.⁴⁻⁶ Because of the rather harsh treatments to which they are exposed, food allergens are generally thought to be more resistant to these processes than other antigens. This is not true of all food allergens, however; and, clearly, food allergens have been identified that are not resistant to these processes. Finally, one must consider intrinsic factors present in foods that seem to affect food allergen development. Why are certain molecules apparently much more allergenic than others? Is this only due to their resistance to various cooking and digestive processes? Clearly, many nonallergenic molecules are equally resistant to food processing methods or digestion, and thus it is

thought that there are other factors present in these food molecules that make them more allergenic. Further studies are clearly needed to elucidate these factors.

Thus, the investigation of food allergens presents unique challenges when compared to studies of other allergens. Exposure to foods is more complicated than that to other antigens in that most food antigen exposure occurs through ingestion, a route that can significantly alter food molecules, resulting in molecules or molecular fragments substantially different from those that are identified in food extracts. It is clear that absorption through the gut is needed to induce a food-allergic response, and thus the ability of molecules to be absorbed through the gut and the role of gut-associated lymphoid tissue, which may be different from that of the central immunological tissue, need to be better understood. The effects on the induction of food allergies of inhalation compared to ingestion of food allergens needs to be better understood. Foods are generally complex, consisting of over 10,000 to 20,000 protein molecules and thus present more of a challenge for study. Finally, it is very difficult, if not impossible, to assess the allergen dose responsible for either sensitizing or provoking a food-allergic response or identify molecular structure of active fragments. Some information is available through food-challenge studies on the amount of food that provokes allergic reactions, but unfortunately nothing is known on sensitizing doses. This, again, is due to the complexity of the induction of food-allergic reactions.³

PROPERTIES OF FOOD ALLERGENS

Although the precise properties that render a food molecule allergenic are unknown, some general characteristics of allergenic food proteins have been identified. Generally, many major food allergens are major food proteins. This suggests that higher doses of a particular allergen enhance the likelihood of producing an allergic response. The fact that most food allergens are usually expressed as percent of total food protein can confuse the issue of exposure doses. For example, when using percent protein as a measurement of a molecule, low-protein foods such as potatoes may contain a protein such as patatin that is 50% of the potato's total protein yet is only a minor potato component (1 g/100 g dry weight), whereas a protein of a similar amount in high-protein foods constitutes a much lower percentage (2–4%) of the total food protein. Clearly, one must take into account the amount of a protein consumed when considering exposure. Food allergens are molecules that must be capable of stimulating immune responses; this is an absolute requirement because molecules that are unable to stimulate antibody production cannot induce an immediate hypersensitivity response. Generally, food allergens are very stable molecules that resist the effects of food processing, cooking, and

digestive enzymes in the gut; intellectually, this makes sense in that molecules that have such abilities would more likely be allergenic. This is not an absolute requirement, however, and there are stable food molecules that are not allergenic, while conversely, several food allergens have been identified that are not highly resistant to these processes.

Food allergens must be polyvalent molecules with at least two or more IgE antibody binding sites. This is because the mast cell or basophil-bound IgE must be cross-linked by these allergen molecules in order to stimulate mediator release. This is an absolute requirement, although one cannot preclude monovalent molecules aggregating *in vivo*. Generally, most food allergens are between 10 and 70 kDa and are primarily glycoproteins with acid isoelectric points; however, this is also the case for most antigens, and thus it is not a distinguishing feature for food allergens.²⁻⁴

The composition of allergenic foods in comparison to nonallergenic foods has always been of interest. The aim, of course, is to be able to identify differences that may be used to identify foods more likely to be allergenic. Comparison of the composition of common allergenic and nonallergenic foods in our diet is summarized in TABLE 1. In general, high-protein foods are more allergenic; of the 23 foods that contain >20% protein, 14 (61%) have been identified as important food allergens. Of the foods that contain <20% protein, only 5/15 (33%) are important allergenic foods. It is interesting to note that of the eight allergenic foods with somewhat lower protein concentration (<30%), 5/8 (63%) have high lipid concentrations (>50%) in comparison to other components; could elevated lipid levels enhance the allergenicity of these proteins? Interestingly, almost all low-protein foods (<20%) have very high concentrations of carbohydrates, with the exception of the tree nuts. Of the 15 foods with <20% protein concentration listed in this table, (omitting tree nuts which have high lipid concentrations) only one (wheat) is considered a major food allergen, and the remaining fruits and vegetables have some allergenic activity but are not major food allergens.

What is the allergen concentration or dose that is necessary to stimulate sensitization to a particular allergen or indeed provoke an allergic reaction? Unfortunately, there is no direct way this can be determined without developing relevant animal models. Perhaps such information could be indirectly obtained by estimating the dose of allergen to which individuals are exposed in relationship to their allergic responses or IgE antibody production (TABLE 2). For this reason, it is very important to express particular protein amounts in terms of dose rather than percent protein, which, as discussed previously, can be misleading.

Cross-reactivity among a number of foods of similar food types as well as between foods and seemingly unrelated substances has been demonstrated. For many years it has been known that foods within a certain group or family can cross-react; examples are members of the legume family, such as soybeans, peanuts, and peas,^{9,10} and crustaceans, such as lobster, shrimp, and

TABLE 1. Composition of common foods in our diet

Food 850.5 g (3 ozs)	Total protein g (%) ^a	Total fat g (%) ^a	Total carbohydrate g (%) ^a	Water g (%) water
Cod, Atlantic fillet, baked	19.39 (96.4)	0.73 (3.6)	0 (0)	64.55 (75.90)
Tuna, light, canned	24.52 (96.1)	1.04 (3.9)	0 (0)	53.4 (62.78)
Shrimp, small, mixed species, steamed	17.78 (95.1)	0.92 (4.9)	0 (0)	65.74 (77.30)
Crawfish, mixed species, boiled	14.88 (93.1)	1.11 (6.9)	0 (0)	68.72 (66.63)
Crab, blue, steamed	17.18 (91.9)	1.51 (8.1)	0 (0)	65.83 (77.40)
Lobster, northern steamed	17.44 (91.6)	0.50 (2.6)	1.09 (5.7)	64.64 (76.00)
Crab, Dungeness crab, steamed	18.97 (91.1)	1.05 (5.0)	0.81 (3.9)	62.34 (66.63)
Salmon, fillet, pink, baked	21.77 (85.3)	3.76 (14.7)	0 (0)	59.28 (69.70)
Chicken breast, boneless, roasted	25.34 (79.3)	6.62 (20.7)	0 (0)	53.07 (62.40)
Tuna, canned	24.75 (78.0)	6.98 (22.0)	0 (0)	50.86 (69.70)
Beef, roast, lean only	23.97 (76.8)	7.23 (23.2)	0 (0)	52.70 (61.63)
Trout, fillet, mixed species, baked	22.62 (75.9)	7.20 (24.2)	0 (0)	53.92 (63.40)
Pork, fresh whole loin, roasted	23.05 (64.8)	12.50 (35.2)	0 (0)	48.90 (57.50)
Lamb, NZ leg, roasted 1/8" trim	21.52 (64.4)	11.91 (35.6)	0 (0)	50.18 (59.0)
Egg	10.63 (52.7)	8.51 (42.2)	1.04 (5.2)	64.04 (75.3)
Soy beverage, fluid	2.34 (42.5)	1.62 (29.5)	1.54 (28.0)	79.35 (93.30)
Soy flour, whole AMI	30.24 (39.0)	30.24 (39.0)	17.01 (22.0)	4.39 (5.16)
Broccoli, boiled	2.64 (36.2)	0.10 (1.4)	4.55 (62.4)	77.14 (90.70)
Milk, whole, cow's*	3.01 (29.1)	3.06 (29.6)	4.26 (41.2)	80.52 (88.0)
Peanuts, dry roasted	20.16 (25.0)	42.27 (52.4)	18.29 (22.7)	1.32 (1.55)
Almonds, dry roasted	18.12 (22.8)	45.25 (56.8)	16.24 (20.4)	1.79 (2.10)
Okra, boiled	1.59 (20.2)	0.14 (1.8)	6.13 (78.0)	76.45 (89.90)
Stringbeans, canned	0.98 (16.4)	0.09 (1.5)	3.83 (64.2)	79.35 (93.30)
Wheat grain, durum	11.65 (15.7)	2.10 (2.8)	60.47 (81.5)	9.27 (10.90)
Brazil nuts, large dried	12.16 (15.3)	56.30 (71.0)	10.89 (13.7)	2.84 (3.33)
English walnuts, dried	12.16 (15.1)	52.65 (65.5)	15.56 (19.4)	3.10 (3.69)
Corn, yellow frozen	2.34 (12.1)	0.37 (1.9)	16.67 (86.0)	65.23 (76.7)
Apple	0.16 (11.9)	0.31 (2.3)	13.1 (96.5)	71.36 (83.90)
Flour, all purpose white enriched	8.79 (11.8)	0.83 (1.1)	64.89 (87.1)	10.12 (11.90)
Eggplant, boiled	0.71 (10.8)	0.20 (3.0)	5.65 (86.1)	78.08 (91.80)
Carrots, boiled	0.93 (9.3)	0.15 (1.5)	8.93 (89.2)	74.33 (87.40)
Pecans, dried halves	6.59 (8.3)	57.49 (72.3)	15.48 (19.5)	4.10 (4.82)
Corn flour, whole grain white	5.89 (7.9)	3.28 (4.4)	65.40 (87.7)	9.27 (10.90)
Potato flour	5.87 (7.6)	0.29 (0.4)	70.68 (92.0)	5.55 (6.53)
Orange	0.80 (7.3)	0.10 (0.9)	10.04 (91.8)	73.82 (86.80)
Banana	0.88 (4.2)	0.41 (1.9)	19.90 (93.9)	63.19 (74.30)
Grapes, Thompson seedless	0.56 (3.5)	0.49 (3.0)	15.14 (93.5)	68.55 (80.60)

^a Percent dry weight.^b Percent total weight.

crayfish.^{11,12} However, these cross-reactivities may have very different clinical endpoints. Cross-reactivity among legumes demonstrated by *in vitro* tests is not necessarily clinically significant.¹³ However, cross-reactivity among crustacea can be clinically significant, and certainly allergy to one crustacean suggests potential reactivity to other crustacea, although not absolute. Cross-

TABLE 2. Food allergen concentration

Protein	% Total protein
Egg white allergens	
Ovalbumin (Gal d 2)	54
Ovomucoid (Gal d 1)	11
Conalbumin (Gal d 3)	12–13
Ovomucin	1.5–3.5
Lysozyme	3.4–3.5
Milk allergens	
β -Lactoglobulin	9
Casein	80
BSA	1
α -Lactalbumin	4
Soybean allergens	
β -Conglycinin (β subunit)	18.5
Kunitz trypsin inhibitor	2–4
Soy lectin	1–2
β -Conglycinin (α subunit)	18.5
Glycinin	51
Gly m Bd 30K	2–3
Peanut allergens	
Vicilin (Ara h 1)	1 ^a
Conglutin family (Ara h 2)	6 ^b
Peanut lectin	1.3
Mustard allergens	
Sin a 1	20
Bra j 1	20
Fish allergens	
Parvalbumin (Gad c 1)	0.22–0.44
Shrimp allergens	
Tropomyosin (Pen a 1)	20 ^c
Potato allergens	
Patatin (Sol t 1)	30–40

^aCrude peanut meal.^bCrude peanut extract.^cCrude shrimp extract.

reactivity can also occur between foods and seemingly unrelated substances, and indeed this has been of greater interest in recent years. Cross-reactivity has been demonstrated with different pollens, particularly birch pollen, with a variety of fruits and vegetables, based on investigations reported primarily in Europe.^{14–17} More recently, with increased interest in latex allergy, cross-reactivity has also been demonstrated between latex proteins and those present in fruits and vegetables.^{18,19} Indeed, these cross-reactivities of fruits and/or vegetables with inhaled allergens have clinical significance in that some patients have developed food allergies subsequent to inhalant exposure. It has been shown by *in vitro* assays that crustacea and mollusks can cross-

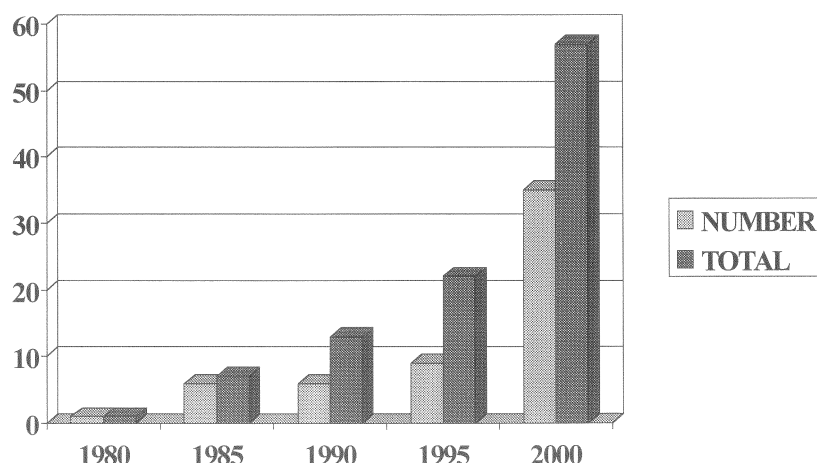


FIGURE 2. Common food allergens identified, sequenced, and cloned from 1980 through 2000. The number of food allergens cloned has increased substantially during the past 20 years, and the results to date suggest that it will continue to increase at a very rapid pace. As of 2000, 57 food allergens had been cloned.

react,²⁰ probably due to the muscle protein tropomyosin, a common allergen present in crustacea. Whether this cross-reactivity is clinically significant or not remains to be determined, since many crustacea-allergic individuals can consume mollusks without reactivity. Indeed, classic IgE-mediated allergic reactions to mollusks with several exceptions^{21–23} have not been well described.

Since 1980, a number of food allergens have been identified and sequenced (FIG. 2). Although common structural properties have been sought, no unique structural feature for food allergen has been identified. Over the years, the number of food allergens identified and characterized have substantially increased. To date, the amino acid sequences of at least 57 food allergens have been determined; based on available technologies, this number will increase substantially over the next several years. More information on allergen structure will provide a more accurate allergenicity assessment, particularly since one approach to characterization of molecules of unknown allergenicity is based on amino acid sequence similarities.^{4,6}

FOOD ALLERGEN EPITOPES: SHRIMP TROPOMYOSIN

The major allergen in shrimp and indeed other crustacea has been shown to be the muscle protein tropomyosin.^{24–28} Tropomyosin is a major (approx-

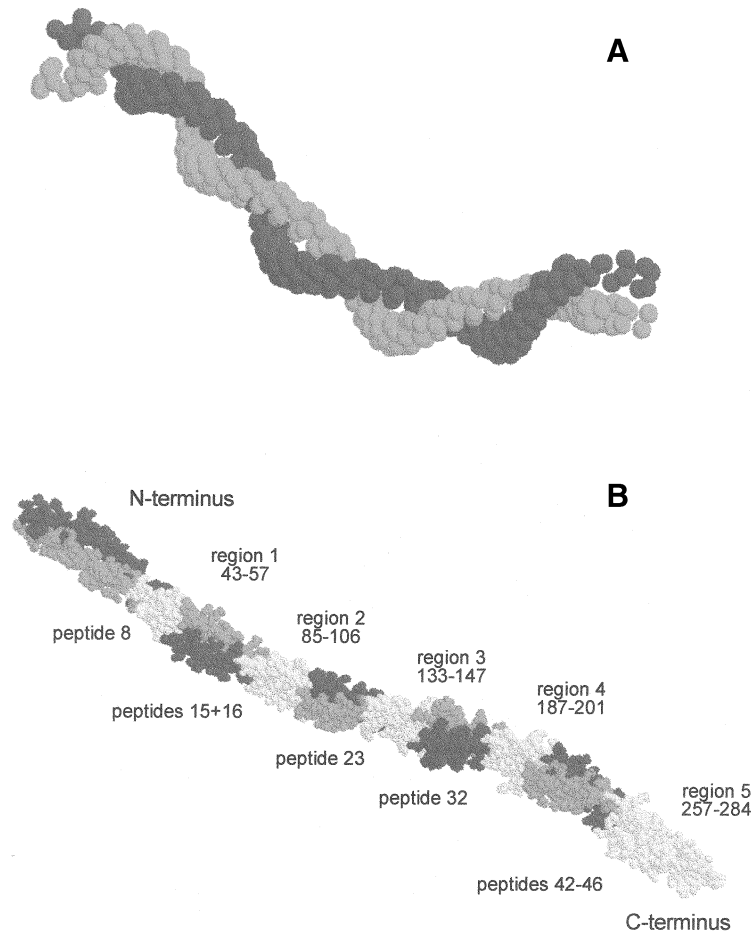


FIGURE 3. Coiled-coil structure of the tropomyosin molecule (**top, A**) and this structure with IgE-binding regions identified (**bottom, B**). Tropomyosin molecule is represented schematically as two chains (red and blue) that are in alpha-helical formation, each chain wound around the other, demonstrating the typical coiled-coil structure of tropomyosin. The structure is typical for all tropomyosin molecules. The same molecule is shown in the bottom portion of the figure (**B**) with the IgE-binding regions highlighted in yellow. Each chain of tropomyosin is identical and parallel. IgE-binding regions appear to exist throughout the entire region portion of the molecule.

imately 20% of the dry weight) shrimp protein, is the only major allergen identified in shrimp, and is thought to be a major allergen in other invertebrates as well.²⁹⁻³² Tropomyosin molecules consist of two identical polypeptide chains, each in an alpha-helical formation coiled around the other; this is

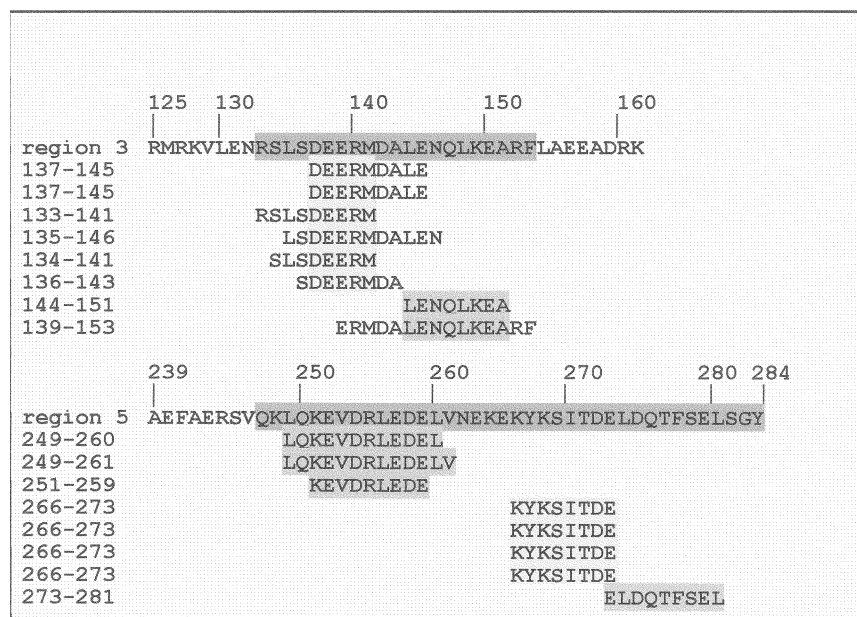
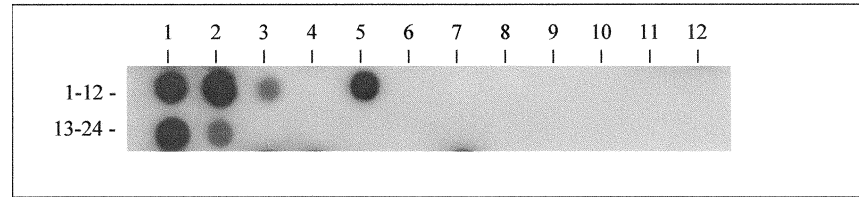
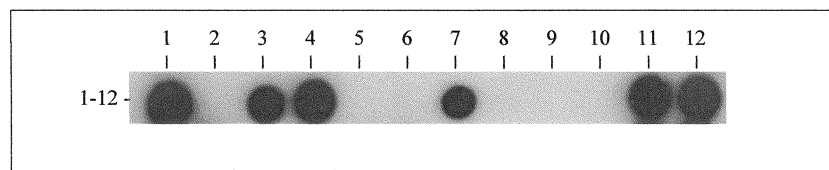


FIGURE 4. Individually recognized epitopes of regions 3 and 5 of the major shrimp allergen, Pen a 1. Epitopes, recognized by individual region-reactive sera, are summarized in the figure. It is interesting to note that the six sera reactive with epitope 3a appear to generally vary considerably in their reactivity, all reacting to a common core of five amino acids. Variability is seen with region 3b as well. In contrast, the variability appears to be reduced for individual sera reacting to epitopes 5a and 5b.

referred to as the coiled-coiled structure of tropomyosin,³³ as illustrated in FIGURE 3. Studies in our laboratory³⁴ have identified five major IgE-binding regions present in shrimp tropomyosin (FIG. 3). The IgE-binding epitopes within these regions have been characterized using synthetic overlapping peptides of 5–15 amino acids in length with an offset of one to two amino acids for a particular region. Region-specific antisera were tested for IgE antibody reactivity, and an epitope was identified as the shortest peptide with maximal IgE binding to that antiserum. Using these results, eight IgE-binding epitopes in five main allergenic regions have been detected.³⁵ An example of epitopes identified in two major regions, region 3 and region 5, is shown in FIGURE 4. Of interest is the fact that there can be significant variability of individual serum reactivity to epitopes. For example, reactivity to epitope 3a demonstrates significant wobble in IgE antibody reactivity with a common core region of five amino acid residues being the same. In epitopes 5a and 5b, the wobble effect is much reduced, and reactivity of individual sera is fairly



Pen a 1 Epitope 5a:KEVDRLEDE (251-259)



Pen a 1 Epitope 5b:KYKSITDE (266-273)

FIGURE 5. Combinatorial substitutions in epitopes 5a and 5b. **Pen a 1 Epitope 5a:** KEVDRLEDGE (251–259) (spot 1); substitution 2 (S for E at position 252), substitution 3 (I for V at position 253), substitution 4 (D for R at position 255), substitution 5 (S for E and I for V), substitution 6 (S for E and D for R), substitution 7 (I for V and D for R), substitution 8 (S for E, I for V, and D for R), substitution 13 (T for E at position 252), substitution 14 (T for E and I for V), substitution 15 (T for E and D for R), and substitution 16 (T for E, I for V, and D for R). **Pen a 1 Epitope 5b:** KYKSITDE (266–273) (spots 1 and 11); substitution 2 (F for S at position 269), substitution 3 (C for T at position 271), substitution 4 (D for E at position 273), substitution 5 (F for S and C for T), substitution 6 (F for S and D for E), substitution 7 (C for T and D for E), substitution 8 (F for S, C for T, and D for E), and substitution 12 (S for T at position 271).

similar. Perhaps this reflects differences in affinity of IgE antibodies to different epitopes and raises the question whether IgE antibody reactivity to certain epitopes may be more relevant clinically than that to other epitopes on the same allergen.

To identify amino acid residues in an epitope essential for IgE antibody binding, mutated peptide epitopes with substituted amino acids based on the amino acid sequence of homologous regions in nonallergenic tropomyosins were synthesized and tested for IgE antibody binding with epitope residue antisera. An example of the IgE antibody binding activity to mutated epitopes 5a and 5b is shown in FIGURE 5. The results indicate that one amino acid substitution may have no effect, reduced or abolished IgE binding, or even enhanced IgE binding.^{35–36} This certainly has significant implications when assessing IgE antibody reactivity to food allergen epitopes. Generally, greater than two substitutions usually abolish IgE-binding ability to modified Pen a 1 peptides. Substitutions from the epitope center are more likely to eliminate (59.5% IgE

binding) as opposed to substitutions on the epitope periphery (39.1%). Most (63.5%) of the single substitutions that eliminate IgE binding are not conservative (different amino acid groups) as compared to the 23.1% of the conservative (same amino acid group) substitutions.³⁵ Whether these observations for IgE binding epitopes of tropomyosin are relevant to those of other food allergens is not clear. Generally, however, our results are consistent with those reported for epitopes of major peanut allergens Ara h 1, 2, and 3.^{37–39} Common patterns of epitope structure and activity observed for food allergens would provide important information for assessment of the allergenicity of food proteins.

PREDICTING FOOD PROTEIN ALLERGENICITY: FOOD FOR THOUGHT

Based on current knowledge of the properties of known food allergens, can the allergenicity of a novel protein to which there is no prior human exposure be precisely predicted? The answer at this time is probably no; however, reasonable assessments based on our current knowledge of food allergens can be made. The information available on known food allergens is increasing, and thus the precision in predicting food protein allergenicity will increase as well. Several important issues are relevant to any type of predictive model. First, food allergen concentration in relationship to food sensitization or provocation of an allergic reaction needs to be determined. Unfortunately, little, if any, information is available on the sensitizing dose of a food allergen other than crude estimates of doses in prior exposures; there is more information, although sparse, on the allergen dose provoking an allergic reaction. Better estimates of exposure levels to known food allergens that stimulate IgE antibody production or provoke allergic reactions will aid development of an indirect measurement of sensitization dose. If sensitizing doses for different food allergens can be estimated and sensitization does not occur below such doses, can a level of food allergens be determined below which sensitization does not occur? Such information, obtained through analysis of the doses to which we are exposed to food allergens, could be invaluable in assessing novel protein allergenicity.

Second, a protein's ability to resist degradation by processing and enzymatic digestion has been used to assess potential allergenic and nonallergenic food molecules; indeed, it has been a criterion for assessing the potential allergenicity of novel proteins. Nevertheless, many food proteins that are not allergenic are stable to processing and enzymatic digestion. Conversely, some protein molecules have been shown to be allergenic yet are sensitive to pro-

cessing and/or enzymatic digestion. Does this mean that stability of molecules is not important in allergenicity assessment? Probably not, but it does tell us that this is not an absolute criterion, and one must be careful in using this information for assessing proteins of unknown allergenicity.

Additional criteria to assess the allergenicity of proteins must be developed. The sensitizing dose for an allergen mentioned previously may be useful in estimating the amount of a food allergen component below which the induction of an allergic response is unlikely. Many high-protein foods such as shellfish are major food allergens; however, there are other high-protein foods that contain similar molecules, such as meats like beef or chicken, that are not very allergenic. These proteins from less allergenic sources are more similar to molecules expressed in man. Thus, the foreignness (immunogenicity) of certain proteins has an effect on allergenicity. For example, crustacea belong to a different phylum than humans, cattle, or pigs; and thus crustacean tropomyosins are more foreign and therefore more allergenic in humans compared to the more closely related beef or pork tropomyosins.

Amino acid sequence similarity is used to compare protein molecules with known allergens; however, the criteria for comparison have not been adequately described. What characteristics are important to compare? Are selected sequence comparison of epitopes (B or T cell) useful or are comparisons of the entire molecule more significant? What length of amino acid sequence and what percent of identity are necessary for a match? Such criteria are particularly important since it has recently been shown that small sequence changes, even in one amino acid, of IgE-binding epitopes in peanut or shrimp allergens may abolish or even enhance IgE antibody reactivity.³⁵⁻³⁹ Thus, under current assessment methods, theoretically an amino acid sequence from a protein of unknown allergenicity may have 90% identity with an allergenic epitope, yet according to current criteria may not be included as a potential risk. Alternatively, should amino acid sequence homology of novel proteins with nonallergens be considered as important? If a protein has a sequence homology of more than 80% with a known nonallergen, does that fact rate importance in allergenicity assessment? Should the similarity or dissimilarity to human proteins (i.e. foreignness) be assessed? All of these issues need further consideration.

Finally, development of animal models that have been adequately validated as surrogates of human IgE antibody responses is required for more precise assessments of the allergenic potential of proteins. Such models must be adequately described with regard to the type of exposure, the route of exposure, the number of exposures, the allergen dose, and the use of adjuvants. My own prejudice is that an animal model that reflects all aspects of food allergy is not only unlikely to be developed, but also probably not necessary for assessment of novel protein allergenicity. However, an animal model of allergenicity, although reflecting only one aspect of food allergy, would serve to provide an important basis for testing or assessing the allergenicity of novel

proteins. For validation, animal models must react to allergenic foods as opposed to nonallergenic foods and recognize protein allergens within the allergenic foods as opposed to nonallergenic proteins within the same foods. This can be easily assessed by Western blotting with animal serum compared to human sera from food-allergic individuals. Finally, epitope reactivity of animal IgE antibodies must be compared to that of humans. One would expect that a validated animal model of allergenicity would produce IgE antibodies that react to epitopes similarly to human IgE antibodies. If all of these criteria correlate with human allergic responses, such a model will be an invaluable tool for the assessment of not only novel proteins but any new proteins to which persons are exposed.

Clearly, our body of knowledge of food allergen structure has increased substantially during the past 20 years. Allergens are molecules that generally (although not always) appear to be major protein components within a particular food and are usually resistant to processing, cooking, and digestion. Further study of the general properties of foods in relationship to their allergenicity is encouraged in that this may provide more useful information on the basis of allergenicity of these molecules. For example, foods of medium protein concentration that are highly allergenic, such as peanuts and tree nuts, appear to have higher levels of fat. This might be useful in protecting allergenic molecules during the digestive process. Furthermore, little information is known about the dose of allergen required to stimulate immune response. Little consideration has been given to homology of allergens in relationship to their foreignness. These are certainly important areas that warrant further study. The evidence to date suggests that we cannot yet precisely predict the potential allergenicity of a molecule from which there is no prior human exposure. However, based on our knowledge of food allergens, it is possible to make educated estimations about the potential allergenicity that should exclude any major new allergens from being introduced into our food supply through genetic modification of foods.

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