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Why are Some Proteins Allergenic? Implications for Biotechnology

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ABSTRACT: In recent years, a number of agricultural crops have been developed with recombinant DNA technology. Because the transferred genes code for proteins that are ordinarily not present in these particular foods, there is concern about the potential allergenicity of these new crop varieties. Foods contain many proteins; however, only a small fraction are allergens. Although the structural properties of proteins that cause allergic reactions have not been characterized completely, known food allergens in general have molecular weights between 10 and 70 kDa, stimulate the immune response (induce the production of allergen-specific IgE), and are stable molecules that are resistant to processing, cooking, and digestion. Although any type of food is potentially allergenic, the majority of food allergies are caused by a small group of foods (cows' milk, nuts, legumes, eggs, seafood). Cross-reactivities occur within a given food group and between foods and seemingly unrelated proteins.

Even though most transgenic foods are considered safe, biotechnological manipulation can affect crop allergenicity. The safety evaluation of transgenic foods is relatively easy when the allergenicity of the gene sources are known. The recombinant food can be assayed using traditional *in vitro* inhibition assays. Recently, reduced allergen content of biotechnologically altered rice was shown. In contrast, increased allergenicity was demonstrated in transgenic soybeans after a methionine- and cysteine-rich protein from Brazil nuts, identified as a major Brazil nut allergen, was expressed in soybean to increase its content of sulfur-rich amino acids. The most difficult issue regarding transgenic food allergenicity is the effect of transfer of proteins of unknown allergenicity. The challenge is to determine whether these proteins are allergenic as there is no generally accepted, established, definitive procedure to define or predict a protein's allergenicity. Comparing the structures of the transferred protein with known allergens and allergen epitopes could be one approach. Additionally, Th-2 cell stimulation, the production of IL-4, and IgE antibody induction in animal models may help to evaluate the potential allergenicity of a protein.

In conclusion, there is no evidence that recombinant proteins in newly developed foods are more allergenic than traditional proteins. The evidence suggests that the vast majority of these proteins will be completely safe for the consumer. The concern is that if a few transgenic foods cause serious allergic reactions, this could undermine the public's confidence in such products. It is essential that proper guidelines are established and tests are developed to assure that this will not occur.

KEY WORDS: recombinant DNA, recombinant proteins, biotechnology, allergenicity, transgenic foods, food safety, food allergy, food allergen.

I. INTRODUCTION

A. Recombinant Proteins in Foods

New plant varieties that can be used as sources of foods, such as fruits, vegetables, grains, and their byproducts, are being developed by using recombinant DNA technologies.¹⁻⁵ Specific genetic modifications based on these technologies

have been made, including the transfer of proteins into plants from other species, which could not ordinarily be done by traditional breeding techniques. Recombinant DNA technologies offer plant breeders the opportunity to gain a number of useful properties, including the increase in major plant constituents¹ that can result in dramatic changes in food composition. In addition, food crops are being developed to resist pests,

diseases, adverse weather and soil conditions, and to tolerate chemical herbicides and improved characteristics for food processing.³ A number of potential foods that have been derived from these new plant varieties by recombinant DNA technologies are approaching commercial introduction.²

Many agricultural crops have been developed with recombinant DNA technology, and at least 30 different varieties are currently being field tested.³ Because some genes governing these new traits code for proteins that are ordinarily not present in that food, there is concern about the effects of the transferred proteins on individuals ingesting them.¹⁻³ A major concern is the potential of these transferred proteins as food allergens.^{2,3}

B. Food Allergy

A variety of adverse reactions can be observed following exposure to foods that can be confused with true food allergy because they have similar symptoms.⁴ An adverse reaction to a food is any clinically abnormal response attributed to exposure to a food or food additive and includes both immunologic and nonimmunologic reactions. Food allergy, or food hypersensitivity, is a specific type of immunologically mediated adverse reaction resulting from the ingestion of a food or food additive. This reaction occurs only in some individuals, requires prior exposure (sensitization), may occur only after a small amount of substance is ingested, and is unrelated to any physiological effect of the food or food additive.⁴ Most food allergies are mediated by IgE antibodies and have a rapid onset, typically within minutes of exposure.⁴ Other adverse reactions to foods are food intolerance, an abnormal physiological response to an ingested food or food additive; food poisoning, which is basically a toxic reaction; and pharmacologic food reactions due to chemicals that produce a drug-like effect. These latter three reactions are nonimmunologic.⁴

Precise figures concerning the prevalence of food allergies are difficult to obtain. In clinical surveys, 2 to 4% of children younger than 6 years have reproducible allergic reactions to foods. Studies in adults suggest that 1 to 2% of the general adult population is sensitive to certain foods or food additives. This contrasts dramatically with

the public's perception of the importance of allergic reactions to foods. It has been reported that at least one of four atopic adults believe that they have experienced adverse reactions following the ingestion or handling of foods. Similarly, parents believe that one of four of their children has experienced at least one adverse reaction to a food.⁴

C. Immunopathologic Mechanisms of Allergy

The induction of allergic hypersensitivity and the effector mechanisms in allergic reactions are summarized in Figure 1. In the induction of an allergic response, allergens that are typically innocuous enter the body via mucosal surfaces and are processed by local antigen-presenting cells that present peptide fragments of the antigen to T lymphocytes. Under conditions (not fully known) that favor a Th2 rather than a Th1 lymphocyte response, recognition between the Th-2 and antigen-specific B cells results in the release of soluble factors such as interleukins 4, 5, and 13 that in turn lead to B cell proliferation and differentiation and the production of allergen-specific IgE.⁵⁻⁸ Antigens that elicit such an IgE response are called allergens. There is some evidence for genetic control of IgE antibody production to specific allergens.^{9,10} IgE binds to specific receptors on mast cells or basophils, thus sensitizing these cells. Subsequently, if an allergen reaches the sensitized mast cell, it cross-links surface-bound IgE, triggering the release of preformed and newly synthesized mediators. These mediators in turn elicit the clinical signs and symptoms of allergic diseases, including asthma, eczema, hayfever, and anaphylaxis.^{11,12}

D. Development of Food Allergy

A number of factors can affect the development of food allergy. One is the amount of a particular food that is ingested, which is influenced by the diet and culture of a country. This can have a significant effect on the prevalence of a specific food allergy in that region. Examples of this are codfish allergy in Norway, rice and soy allergy in Japan, and peanut allergy in the U.S.

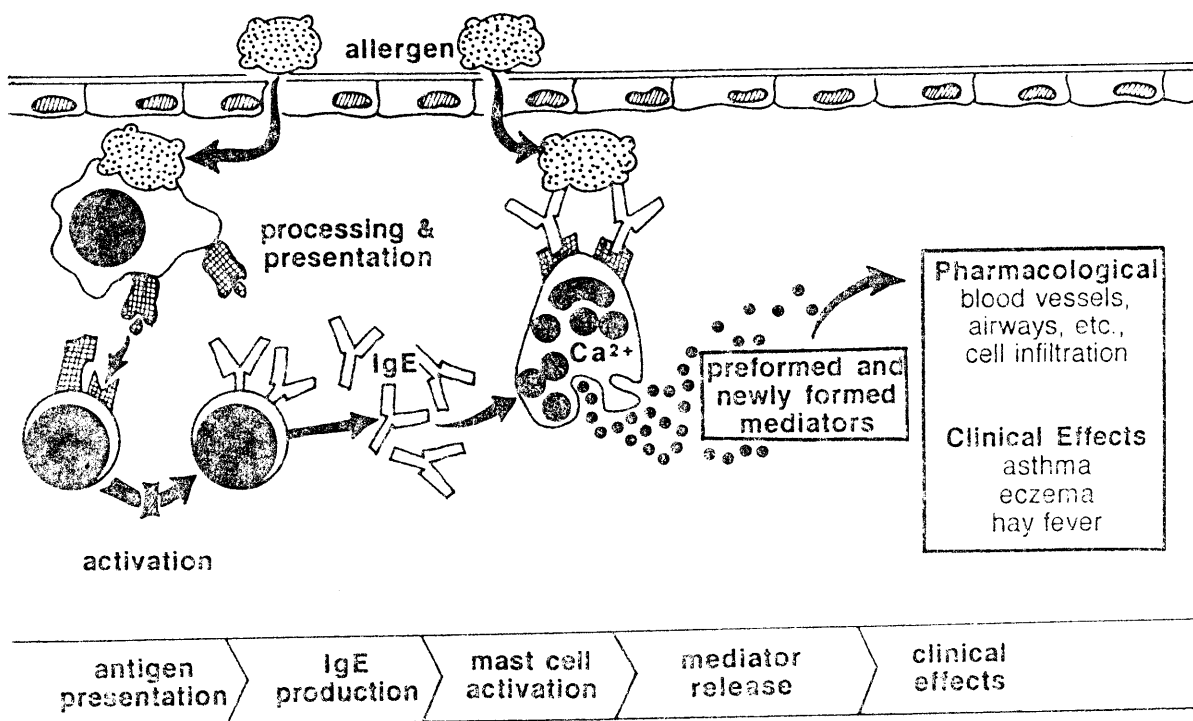


FIGURE 1. Induction and effector mechanisms in IgE-mediated hypersensitivity. Allergen, generally an innocuous protein molecule, after crossing mucosal membrane barriers can stimulate IgE antibody production. Subsequent interaction of the allergen with mast cell or basophil-bound IgE antibodies leads to the release of mediators that are potent pharmacological molecules that result in the clinical effects of allergy.

Route of exposure is another important factor in the development of a food allergy. Not only are food allergens ingested, but exposure can also occur through skin contact and inhalation.¹³ Gut permeability has long been thought to be a major factor in food allergy sensitization. Actually, however, there has been very little investigation of this phenomenon. Processing of food is another factor that can substantially alter food allergen content. Finally, intrinsic factors are present: some foods apparently are very allergenic, while other foods are rarely if ever allergenic.

II. PROPERTIES OF FOOD ALLERGENS

A. General Characteristic of Allergenic Food Proteins

Foods contain a wide variety of proteins, yet only a few are known allergens. In plant foods such as soybeans and peanuts, many of the aller-

gens appear to be storage proteins¹⁴⁻¹⁷ that are present in large amounts in these particular foods. It is tempting to infer that the likelihood of allergenicity correlates with the degree of exposure to a particular protein. However, proteins that occur only in minor amounts in foods can also be major food allergens. For example, Gad c 1, the major allergen in codfish, is not a predominant protein in codfish.¹⁸ Also, proteins that are major components of many foods, such as actin, myosin, and tropomyosin from beef, pork, and chicken, are not allergenic. Thus, while the exposure dose is likely a critical factor, the inherent immunogenicity of the protein is clearly more important.

As with all antigens, food allergens must be foreign molecules to stimulate a significant immune response because molecules of host origin do not normally elicit antibody production. Most food allergens are stable molecules that resist the effects of food processing and cooking, and the digestive processes.¹⁹ There are, however, exceptions such as the labile allergens present in apple

and other fresh fruits and vegetables.^{20,21} Generally, such labile allergens cause reactions that are restricted primarily to the oral mucosa, as they rapidly lose allergenic activity during digestion. Conversely, it has been suggested that digestion can alter antigens by releasing or uncovering new epitopes, thus increasing allergenicity. This has not been well investigated to date.

A prerequisite for any allergen is the ability to stimulate an immune response. Why certain proteins do this better and are more allergenic than others is not yet understood. Allergens triggering allergic reactions must be polyvalent, that is, have several epitopes that bind IgE antibodies because allergens must bridge IgE molecules on the surface of mast cells to initiate degranulation; thus, they are somewhat constrained in molecular dimensions. Most known food allergens have molecular weights between approximately 10 and 70 kDa. However, proteins larger than 70 kDa can be allergens. For example, Ara h 1, the major peanut allergen, probably exists naturally as a very large protein (150 to 200 kDa or more). Its described 66-kDa size is based on SDS dissociation into subunits. Although smaller molecules could conceivably act as haptens, a molecular weight of 10 kDa probably represents the lower limit for an immunogenic response. The upper limit for molecular size is probably the result of restricted mucosal absorption of larger molecules. Generally, food allergens are glycoproteins with an acid isoelectric point. However, many nonallergenic proteins also share these properties. While there are some exceptions, clearly the most potent food allergens are stable molecules that are resistant to processing, cooking, and digestive enzymes while they remain soluble and absorbable through the intestinal tract.

The portions of an antigen/allergen molecule that interact with cells of the immune system or antibodies are defined as epitopes. Those epitopes that react with T cells are called T-cell epitopes.²² Epitopes that react with antibody or antibody-producing B cells are called B-cell epitopes.²³ Whether all B- and T-cell epitopes are different from each other is still controversial and may vary from allergen to allergen.

Epitopes are either conformational or linear. Conformational epitopes depend on the tertiary

structure of a protein and may be composed of several adjacent amino acid sequences on the surface of the protein. Linear epitopes are composed of only the amino acid sequence itself. It is generally thought that T-cell epitopes are linear, whereas B-cell epitopes are conformational. However, there may be exceptions to this rule.²³ An epitope composed of a series of covalently linked amino acids is called a continuous epitope. An epitope that is composed of two adjacent noncontinuous amino acid sequences is called a discontinuous epitope. A schematic diagram of an allergen molecule with a continuous and discontinuous epitopes is shown in Figure 2. For continuous epitopes, the minimum number of amino acid residues is six. Discontinuous conformational epitopes have a minimum of 16 amino acid residues.²³

T-cell epitopes are generally small peptide fragments (12 to 18 amino acids) present in a linear sequence of an allergen. T-cell epitopes are recognized by specific T-cell surface receptors (TCR) only after the antigen has been phagocytized, processed, and presented by an antigen-presenting cell (APC). Determining the complete repertoire of T-cell epitopes of an allergen is a formidable task due to the large number of epitopes and the variable recognition of epitopes by different individuals. Characterization of T-cell epitopes has only begun to be attempted for food allergens.

B. Multiple Allergens

Most allergenic foods contain more than one significant allergen. In an attempt to organize allergens with regard to patient reactivity, the following classification system has been developed to better describe allergens. Allergens to which the majority (>50%) of patients sensitive to that food react are described as "major allergens", in contrast to "minor allergens" in the food to which a minority of the patients react.²⁴ Generally, although not always, major allergens tend to be predominant components in the allergen extracts. There have been several attempts to define major allergens based on the number of individuals who react with these materials or on the intensity of their biological reaction.²⁵⁻²⁸ Although

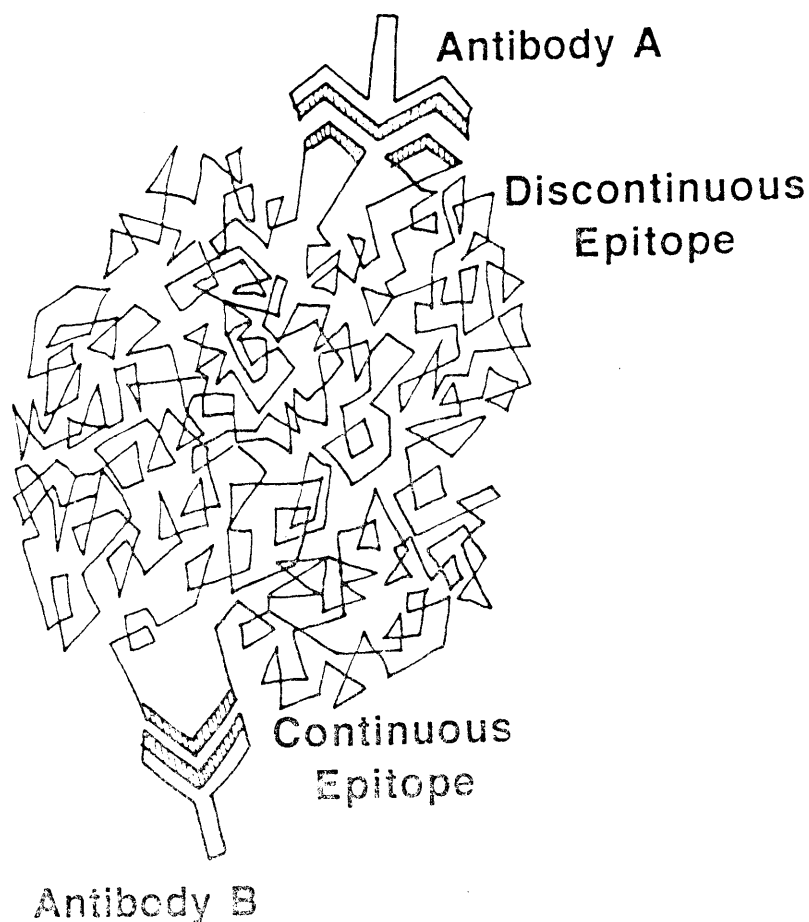


FIGURE 2. Examples of continuous and discontinuous epitopes of a protein allergen molecule. Antibody B reacts to a continuous epitope, whereas antibody A reacts to a discontinuous epitope.

major allergens are generally of greatest interest, we cannot ignore minor allergens that can cause serious reactions in sensitized subjects.

In addition, some allergens occur as a group of proteins having very similar physical, chemical, and immunochemical structures, but which differ slightly from one another in their isoelectric points.^{24,25} The term "isoallergens" has been used to denote such molecules isolated from the same specific source. At least 67% of the amino acid residues in the sequences of two allergens must be identical for the proteins to be isoallergens.²³ The slight differences observed in these allergens are due to minor structural differences in the carbohydrate moiety of the molecule (degree of glycosylation), differences in degree of protein amidation, or genetic variation.²⁵

C. Allergen Nomenclature

Specific designations may be assigned to thoroughly characterized allergens according to the accepted taxonomic name of their source. The first three letters of the genus and the first letter of the species are used to indicate the source. An Arabic number is assigned according to the order of their identification; however, generally, the same number is used to designate homologous allergens.^{29,30} For example, the first allergen described in brown shrimp, *Penaeus aztecus*, is designated Pen a 1, and the homologous molecule from the Indian shrimp *P. indicus* is named Pen i 1. Examples of major food allergens identified and their names are shown in Table 1. The nomenclature system also provides rules for

describing allergen genes, mRNAs, cDNAs, and, most importantly, recombinant and synthetic peptides of allergenic interest.³⁰ Examples of the use of this nomenclature system for describing several of the major food allergens are illustrated in Table 1.

III. FOOD ALLERGENS

A. Common Food Allergens of Plant Origin

Theoretically, any type of food can cause allergic reactions. However, reactions caused by cow's milk, nuts, eggs, legumes, and seafood are most frequently described in the literature. Foods of plant origins that cause allergic reactions include those from grains such as rice, rye, and wheat, legumes such as peanuts and soybeans, seeds and nuts, fruits, vegetables, spices, and miscellaneous substances such as honey, fungi,

yeast, cocoa, coffee, and tea.⁴³ The best studied of the major food allergens derived from plants are those from peanut, soybean, and rice.

B. Cross-reactivity Among Food Allergens

Cross-reactivity can occur among food allergens within a given food group. For example, in clinical practice, shrimp-allergic patients report reactivity to other crustacea such as crab, lobster, and crawfish.⁴⁴ Cross-reacting allergens among crustacea have been confirmed by *in vitro* immunochemical studies.^{45,46} Fewer reports exist of cross-reactivity within vegetable food groups; however, one well-studied plant group is the legumes. Significant cross-reactivity, based on *in vitro* assays, is observed among peanuts, garden peas, soybeans, and chickpeas.^{47,48} In contrast to crustacea sensitivity, the clinical relevance of legume cross-reactivity has not been resolved

TABLE 1
Major Food Allergens Identified

Allergen source	Allergens (systematic and original names)	MW (kDa)	Sequence data	Ref.
<i>Gadus callarias</i> (cod)	Gad c 1; allergen M	12	C	31
<i>Gallus domesticus</i> (chicken)	Gal d 1; ovomucoid	28	C	32, 33
	Gal d 2; ovalbumin	44	C	
	Gal d 3; conalbumin (Ag22)	78	C	
	Gal d 4; lysozyme	14	C	
<i>Penaeus aztecus</i> (brown shrimp)	Pen a 1; tropomyosin	36	P	34-35
<i>P. indicus</i> (indian shrimp)	Pen i 1; tropomyosin	34	P	36
<i>Metapenaeus ensis</i> (greasyback shrimp)	Met e 1; tropomyosin	34	C	37
<i>Arachis hypogaea</i> (peanuts)	Ara h 1	63.5	P	16, 17
	Ara h 2	17	P	
<i>Oryza sativa</i> (rice)	Ory s 1	16	C	38, 39
<i>Brassica juncea</i> (oriental mustard)	Bra j 1; 2S albumin	14	C	40
<i>Hordeum vulgare</i> (barley)	Hor v 1; BMAI-1	15	C	41
<i>Sinapis alba</i> (yellow mustard)	Sin a 1; 2S albumin	14	C	42

^a References refer to those where partial (P) or complete (C) sequence data are available.

because clinical hypersensitivity to one legume may not necessarily mean hypersensitivity to all legumes.⁴⁹

In addition to the cross-reactivity of foods within the same group, cross-reactivity has been described between foods and substances that are botanically unrelated or only distantly related. For example, it has been reported that ragweed pollen cross-reacts with melons and bananas;^{50,51} grass and mugwort pollens cross-react with celery and a variety of vegetables;⁵²⁻⁵⁴ and birch pollen cross-reacts with a number of fruits.^{54,55} Studies from our laboratory have shown that marine animals that even belong to different phyla, such as oysters and crustacea⁵⁶ or clams and shrimp,⁵⁷ cross-react.

How can proteins from such distantly related substances cross-react? One explanation may be common structural or functional proteins. For example, profilins are cytoskeleton proteins that are ubiquitous and have a high degree of sequence homology.^{58,59} Based on their recent studies that showed that profilins of various sources have similar allergenicity, Kraft and Valenta proposed profilins as a pan allergen.⁶⁰ Contrary to these findings is the result of our studies and those of other groups of the major shrimp allergen tropomyosin, a major muscle protein.³⁴⁻³⁷ Although shrimp tropomyosin is a major allergen, tropomyosin in beef, pork, and chicken are not allergenic even though they have at least 60% amino acid sequence homology with shrimp tropomyosin. Thus, one must be careful when inferring cross-reactivity from common amino acid sequences of similar proteins. The clinical significance of cross-reacting allergens is not entirely clear.⁶¹ However, the demonstration of cross-reacting allergens in different plants and animals poses serious concerns about the cross-reactivity of known allergens with protein products developed by recombinant DNA technologies.

IV. BIOTECHNOLOGY OF FOODS

A. Allergenicity of Recombinant Proteins

cDNA clones have been isolated for a number of allergens in recent years. These are mostly

inhalant allergens such as grass pollen and the fungi *Alternaria*, *Aspergillus*, and *Cladosporium*.^{62,63} Recombinant *Aspergillus* allergen is one of the few cases in which the recombinant allergen induces a positive skin test in allergic subjects. Molecular cloning of stinging insect (Hymenoptera) venom proteins has also been reported. Although primary emphasis has been directed at inhalant allergens, cDNA for several food allergens have also been cloned and expressed as recombinant allergens.⁵⁶⁻⁵⁹ Two recombinant peanut allergens, Ara h 1 and Ara h 2, have been expressed.^{16,17} cDNA cloning has been reported for a major allergenic protein from rice.^{38,39} Monslave et al.⁴⁰ have also described the cloning and expression of a major allergen from yellow mustard seed (Sin a 1).

Mena et al.⁴¹ cloned the cDNA coding for a major barley allergen associated with baker's asthma. This protein was identified as a glycosylated monomeric α -amylase inhibitor. IgE-binding studies were not performed, however. Recently, Leung and co-workers reported cloning the major allergen from the shrimp *Metapenaeus ensis*.⁵⁷

B. Biotechnologically Altered Foods

When considering the potential effects of biotechnology on food allergenicity, three major issues must be reviewed. The first is the potential ability to suppress or reduce allergen production in a particular food (i.e., genetically engineering hypoallergenic foods). The second major issue is the transfer of known allergenic proteins into new foods. This was shown dramatically by the transfer of a gene for a rich methionine and cysteine protein from Brazil nut to soybeans when the expressed protein was shown to be one of the major allergens of Brazil nut. The third issue is the effect of transfer of unknown protein allergens into new foods. This is the most difficult potential effect to monitor because the target population is unknown, and, in fact, may not develop sensitivity to the new product until after natural exposure occurs. The first issue has a beneficial effect in that foods eliciting allergenic responses can be rendered hypoallergenic through deletion of the allergen or modification of the allergenic

epitopes. This is potentially a very useful effect because it can substantially reduce a food's allergenic activity.

Matsuda and colleagues reported, in a series of studies, the genetic engineering of hypoallergenic rice.^{38,39,64,65} This group recently isolated a rice seed protein of approximately 16 kDa that was identified as a major allergen in rice. A cDNA clone encoding this protein was isolated from a cDNA library prepared from maturing rice seeds. Based on the nucleotide sequence of the cDNA, an antisense RNA strategy was applied to repress expression of this allergen in maturing rice seeds. Seeds from transgenic plants with the antisense gene have substantially lesser amounts of the allergen relative to the wild-type control. These studies are very exciting because they demonstrate that biotechnology can be used in a beneficial way by deleting or suppressing the expression of major allergens.

C. Measurement of Known Allergens by *In Vitro* Assays (IgE Binding)

The transfer of recombinant proteins with known allergic activity into different foods is the least difficult issue to address. To date, most experience with recombinant allergens has been obtained from recombinant inhalant allergens, as described above. For the most part, these recombinant allergens bind IgE antibodies and thus retain their IgE-reactive epitopes. These findings suggest that recombinant proteins expressed in other species (i.e., altered food crops) will probably retain their allergenic activity and must be considered allergenic unless proven otherwise. Can such allergenic recombinant proteins be detected in new foods?

Recombinant allergens in genetically engineered or altered foods can be identified using traditional *in vitro* assays such as the RAST inhibition or the ELISA inhibition assays. Both assays are based on the competitive binding of IgE antibodies in patient serum to the test sample compared with the solid-phase allergen. Testing increasing inhibitor concentrations can be used to measure unknown allergen quantities in assay samples.

These methods are well established, specific, sensitive, and reproducible. The only reagent of limited quantity is patient serum. However, once sensitized individuals are identified, substantial quantities of sera can be obtained and serum pools produced. At least 1 l of plasma can be obtained from sensitized individuals by plasmapheresis. This can provide sufficient reagents for almost unlimited allergen assays. An alternate possibility would be to use monoclonal antibodies raised against such allergens. This would provide a potentially unlimited quantity of the reagent. However, monoclonal antibodies may not necessarily bind to IgE-reactive epitopes, and the only definitive way to identify immunologically active allergens is through demonstration of an IgE-reactive epitope.

The detection of recombinant allergen in a potential food product under development was reported recently by Taylor's laboratory.⁶⁶ They investigated a protein found in Brazil nuts that was expressed in experimental transgenic soybeans. Their study suggested that the Brazil nut protein bound IgE from Brazil nut-sensitive individuals, making it a potential allergen in sensitized subjects. The problem thus created is of a Brazil nut-sensitive subject consuming a soy product without realizing that it contains Brazil nut allergens, and provoking anaphylaxis. These transgenic soybeans have not been commercialized and, if ever commercialized, will contain proper labels. This example illustrates that allergens transferred to other plant species can be detected.

D. Predicting the Allergenicity of Recombinant Proteins of Unknown Activity

The third point, the transfer of proteins of undetermined allergenicity (e.g., from species not usually consumed as foods) into potential foods, poses a much more difficult problem. Are these proteins expressed in such a fashion that they have substantial allergenic activity? Do they contain epitopes that cross-react with known allergens? Predicting the potential allergenicity of these proteins will be a major challenge for the food

industry. To assess proteins of unknown allergenic activity, one can determine the physicochemical properties of these proteins compared with those of major food allergens. However, a definitive answer can only be based on IgE antibody reactivity. This is extremely difficult, if not impossible, to establish if the IgE antibody reactivity is not known. One potential solution is to compare the amino acid sequence of the recombinant protein with those of known allergen epitopes as they become available. The amino acid sequences of more and more allergenic epitopes are being determined and are available in computer data banks. However, because substantial sequence similarities can exist between homologous allergenic and nonallergenic molecules (e.g., tropomyosin),³⁴⁻³⁷ any similarities, of course, must be confirmed by IgE antibody reactivity using *in vitro* assays.

It has been established that a Th2-type lymphocyte response is an integral part of the induc-

tion of IgE antibody synthesis. Thus, a novel approach to assess allergenicity of a recombinant protein might be to measure its ability to elicit a Th2-type response and cytokine production. An experimental approach that might be useful is to determine the ability of recombinant food proteins to stimulate IgE antibody production in an animal model compared with that of known allergens.

IV. SUMMARY AND CONCLUSIONS

Food allergens are almost exclusively protein molecules that can stimulate IgE antibody responses. Although it is suggested that food allergens may contain unique properties, the basis of their allergenicity has not yet been determined. Clearly, recombinant proteins can be allergenic. Measurement of the allergenicity of recombinant proteins is possible. A systematic strategy to assess whether a recombinant protein has allergenic

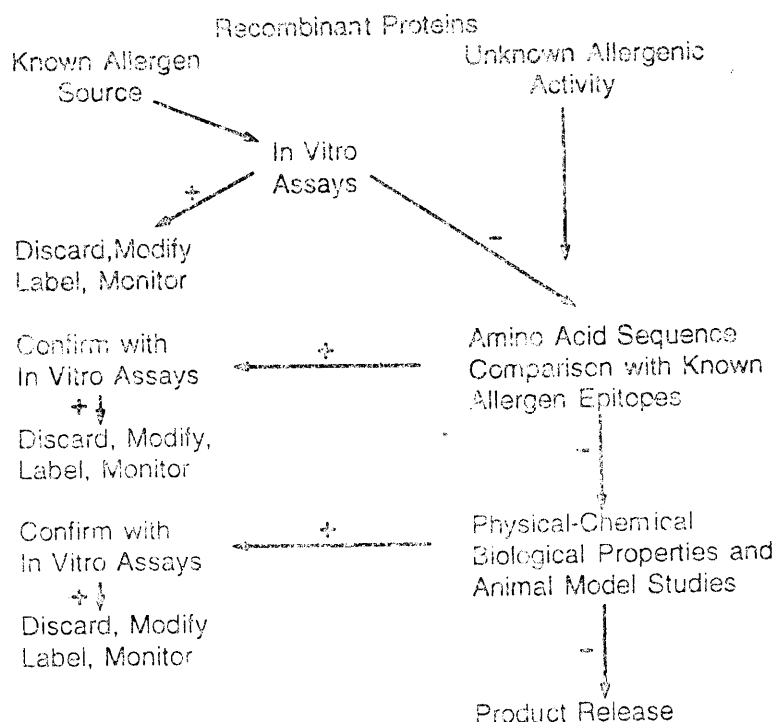


FIGURE 3. Recombinant food proteins — allergen risk assessment. Recombinant proteins of known allergens can be easily tested for allergenic activity by established *in vitro* immunochemical assays. Recombinant proteins from unknown allergenic sources pose a more difficult problem. Several biochemical and immunochemical tests can be used to assess their allergenicity.

activity is summarized in Figure 3. When the protein is a suspected allergen, or comes from a source that contains known allergens, standard *in vitro* assays using the patient's IgE antibodies can be used to determine if the protein is allergenic. Such assays and the required reagents are developed and available. The only limiting factor is sera from allergic subjects.

The main problem is assaying recombinant proteins from sources with undetermined allergenic activity. There is no definitive way to conclusively establish whether these proteins are allergenic. Useful approaches include comparison of their properties with those of known allergens, comparison of their amino acid sequences with those of known allergen epitopes, and measurement of their ability to stimulate IgE antibody responses in animal models. However, any suggested allergenicity must be confirmed by *in vitro* assays using human IgE antibody reactivity. Such approaches should help avoid potentially serious allergic reactions to recombinant proteins among consumers.

There is no evidence that recombinant proteins will be any more allergenic than traditional proteins, and most evidence suggests that the vast majority of recombinant proteins in "new foods" will be completely safe for the consumer. The concern is the few recombinant proteins that could cause serious allergic reactions and thus undermine public confidence in recombinant protein-containing food products. Therefore, it is incumbent upon the food industry, food scientists, and food regulators to develop proper guidelines and tests to ensure that this never occurs.

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