Allergenic Potential of Recombinant Food Proteins

by Carol O'Neil, Gerald Reese & Samuel B. Lehrer

Recombinant DNA technology is being widely used to improve a variety of agriculturally important crops. As genetically engineered crops enter the marketplace, however, there is consumer concern about their safety. Potential allergenicity of transgenic foods is a major concern. Since exposure of food-sensitive individuals can lead to fatal anaphylaxis, detection of allergenic proteins transferred into the food supply is crucial. There are two important components when one would like to test the potential allergenicity of transgenic foods: (1) The transfer of proteins from known allergens into newly developed plant varieties; assessment of the allergenicity of these proteins is relatively straightforward and is done by using *in vitro* tests, including RAST, RAST inhibition, and immunoblotting, and sera from subjects sensitized to the parental variety. (2) The transfer of proteins from foods of unknown allergenicity presents a much more difficult problem. Although there are no definitive models available to predict the allergenicity of proteins, this review describes approaches to assess the potential allergenicity of these proteins and stresses the importance of confirmation of allergenicity by IgE reactivity, whenever possible. Lastly, future directions for bioengineering of foods, particularly the development of hypoallergenic foods, are discussed.

Introduction

Recombinant DNA technology is being used in agriculturally important crops to improve their resistance to disease, herbicides, insects, or environmental insults, to delay ripening, to provide male sterility, to modify starch and oils, or to increase major components of the plant. To date, more than 60 different plant species have been successfully genetically engineered. This field is rapidly expanding, and more than 20 new plant products are expected to be introduced into the marketplace within the next 5 years (see Table 1). Since the traits introduced result from the expression of proteins that are not necessarily part of the recipient species' original genome, there is justifiable consumer concern about the safety of transgenic foods as they enter the marketplace. One major concern is the potential allergenicity of these proteins [1–3].

Food Allergy and Food Allergens

Overview

Plants express at least 100,000 different proteins. Thus, plants contain numerous potential allergens. Theoretically, any food can elicit an allergic response; practically, only about 100 different foods have been documented as causing allergic reactions — and of those only a few foods or food groups (peanut, tree nuts, milk,

TABLE 1
FOODS DERIVED FROM NEW PLANT VARIETIES DERIVED THROUGH RECOMBINANT DNA TECHNOLOGY — FINAL CONSULTATIONS UNDER FDA'S 1992 POLICY

Product	Company	Year of FDA Evaluation
Insect Protected Corn	Dekalb Genetics Corp.	1997
High Oleic Acid Soybean	Dupont	
Modified Fruit Ripening Tomato	Agritope Inc.	1996
Glufosinate Tolerant Corn	Dekalb Genetics Corp.	
Sulfonylurea Tolerant Cotton	Dupont	
Insect Protected Potato	Monsanto Co.	
Insect Protected Corn	Monsanto Co.	
Glyphosate Tolerant/Insect Protected Corn	Monsanto Co.	
Insect Protected Corn	Northrup King	
Male Sterile/Fertility/ Restorer Oilseed Rape	Plant Genetic Systems	
Male Sterile Corn	Plant Genetic Systems	
Glufosinate Tolerant Canola	AgrEvo Inc.	1995
Glufosinate Tolerant Corn	Agr Evo Inc.	
Laurate Canola	Calgene Inc.	
Insect Protected Corn	Ciba-Geigy Corp.	
Glyphosate Tolerant Cotton	Monsanto Co.	
Glyphosate Tolerant Canola	Monsanto Co.	
Insect Protected Cotton	Monsanto Co.	
Virus Resistant Squash	Asgro Seed Co.	1994
Flavr Savr Tomato	Calgene Inc.	
Bromoxynil Tolerant Cotton	Calgene Inc.	
Improved Ripening Tomato	DNA Plant Technology	
Glyphosate Tolerant Soybean	Monsanto Co.	
Improved Ripening Tomato	Monsanto Co.	
Insect Protected Potato	Monsanto Co.	
Delayed Softening Tomato	Zeneca Plant Science	

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eggs, fish, crustacea, and wheat) account for more than 90% of allergic reactions [4]. It is difficult to estimate accurately the prevalence of food allergy. At least one in four atopic adults and the parents of one in four children believe that they have experienced an allergic reaction to food. These figures contrast sharply with the actual prevalence of food allergy. Some 2–4% of children under the age of 6 and up to 2% of adults have reproducible allergic reactions to food. This discrepancy between the perceived and actual prevalence of food allergy has important implications for consumer acceptance of transgenic foods.

Our knowledge of the structure of food allergens is limited compared to the wealth of information available on inhalant allergens. Most food allergens characterized to date (reviewed in ref. [4]) are stable and resist the effects of processing, cooking, and digestion. Further, food allergens are usually glycoproteins with an acid isoelectric point. Like other allergens, food allergens are polyvalent; that is, they have several epitopes that bind IgE antibodies. Most known food allergens have a molecular weight between 10 and 70 kDa. The upper size limit of a food allergen is probably dictated by limitations of mucosal absorption. Despite these similarities, which are shared by many nonallergenic proteins as well, it is not clear why some proteins can provoke an IgE-mediated reaction and others cannot. Clearly, there are as yet undefined intrinsic factors that confer the ability of proteins to stimulate IgE reactivity.

Cross-Reactivity among Allergens

Cross-reactivity has been documented among groups of allergens, including food allergens [4]. For example, some shrimp-allergic individuals also report sensitivity to crab, lobster, or crawfish. *In vitro* tests have confirmed the presence of cross-reacting crustacea allergens. Cross-reactivity has also been reported among vegetable groups, particularly legumes. It is particularly important to note that cross-reactivity has also been confirmed among allergens from disparate groups, notably between ragweed pollen and melons or bananas; grass or mugwort pollens with celery and other vegetables; and mollusks and crustacea. Although the clinical significance of cross-reacting allergens is not always clear, particularly among phylogenetically unrelated groups, their occurrence poses potentially serious concerns about cross-reactivity among known allergens and proteins produced in foods using recombinant DNA techniques [1, 3].

How can proteins from seemingly unrelated groups cross-react? More importantly, how can this be used to help assess potential allergenicity of transgenic foods? One answer may lie in the presence of common structural or functional proteins. For example, profilins, ubiquitous cytoskeletal proteins, have a high degree of sequence homology. Profilins from various sources have been reported to have similar allergenicity [5]. Tropomyosin, a major contractile protein present in all animal species is probably the major crustacea allergen, and cross-reactivity has been demonstrated among tropomyosins from a number of inve brates [6]. On the other hand, tropomyosins from beef, pork, chicken have not been shown to be as allergenic as the tropo osin from shrimp, despite the fact that they share at least a 6 sequence homology.

Identifying Allergens in Genetically Engineered Foods

Overview

Accidental exposure of food-sensitive individuals to spec ic food allergens has lead to numerous instances of fatal or ne fatal anaphylaxis. Therefore, detection of allergenic prote transferred into the food supply is crucial when assessing overall safety of bioengineered foods or food products. Rece ly, strategies to assess the allergenic potential of genetically gineered foods have been developed [1–3]. These strategies based on our current knowledge of the properties and struct of food allergens.

There are two considerations when evaluating potential all genicity of transgenic foods. The first is the transfer of protei from known allergens into newly developed recombinant pl varieties; the second is transfer of proteins from a source unknown allergenicity.

Assessment of the Allergenicity of Genetically Engineered Foods Derived from Known Allergens

Although not without caveats, it is relatively straightforwa to evaluate the allergenicity of genetically engineered foods d rived from plants known to be allergenic. Using the parental v riety as a positive control, *in vitro* immunologic assays can be em ployed to assess the IgE reactivity of transgenic foods. RAST an RAST inhibition and/or ELISA are the tests of choice to quant fy possible differences in total allergen content and allergen com position. Immunoblotting, although less quantitative than oth solid-phase assays, can also be used to identify allergens an compare IgE reactivity to individual allergens. Positive respons in these assays provide strong evidence that an allergen has bee transferred. In that case, the transgenic food would either not b introduced into the marketplace or be required to carry appropr ate labelling with monitoring of any adverse reactions.* No fu ther testing would be required.

If results from the *in vitro* testing are negative or equivoca more definitive *in vivo* testing — skin prick tests and double blind placebo-controlled food challenge (DBPCFC) — can b performed. Again, a positive reaction would mandate labeling o the product, whereas a negative response would be indicative tha the transferred gene did not encode an allergenic protein an product labeling would not be required.

* When transferring proteins from a known allergen, the Food and Drug Administration (FDA) "considers it prudent practice for the producer ini tially to assume that the transferred protein is the allergen. Appropriate *in vitro* or *in vivo* allergenicity testing may reveal whether food from th new variety elicits an allergic response in the potentially sensitive population (i.e., people sensitive to the food in which the protein is ordinaril found)." Further, "labelling of foods newly containing a known or suspect allergen may be needed to inform consumers of such potential." Th source of the original food from which the gene was transferred should appear on the label [7].

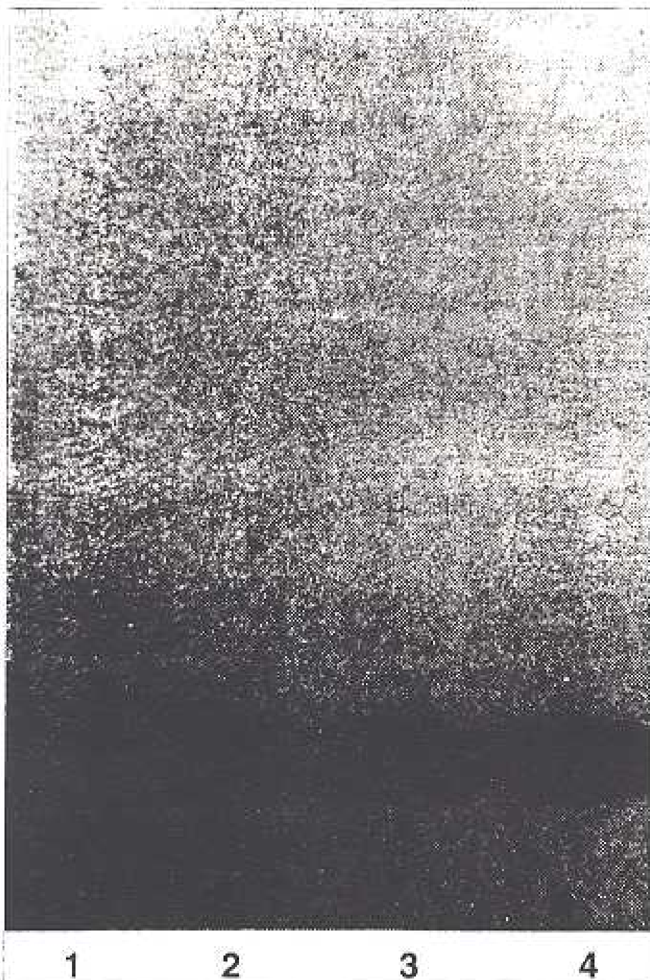

Figure 1. Reactivity of Brazil nut allergenic serum to a 2 S albumin Brazil nut allergen. Lane 1: non-transgenic soybean; lane 2: non-transgenic soybean; lane 3: Brazil nut extract; lane 4: 9 kD 2S albumin Brazil nut allergen. This figure was reproduced from an article by Nordlee and colleagues in *N Engl J Med* 1996; 334:688–692. Reprinted by permission of *The New England Journal of Medicine*, Massachusetts Medical Society.

One foreseeable problem to this approach is the difficulty in obtaining a sufficient number of serum samples, in adequate quantities, from individuals whose clinical sensitivity has been confirmed for use in the RAST or ELISA. Metcalfe and associates recommend the use of 14 sera from individuals with sensitivity confirmed by DBPCFC [3]. Unfortunately, it may be difficult, if not impossible, to identify such a relatively large number of sensitive subjects, especially for less common food allergens. However, if such individuals can in fact be identified, substantial amounts of sera could be obtained and serum pools could be produced for use in subsequent testing. An alternative would be to use allergen-specific monoclonal antibodies that can be produced in virtually unlimited quantities. However, these antibodies do not necessarily react with IgE-binding epitopes, so that reactivity would suggest only common structural features.

Examples of Allergen Detection in Transgenic Foods

The importance of testing recombinant proteins for allergenicity has been clearly demonstrated. For example, the methionine-rich 2S storage protein from Brazil nut was expressed in soybean to improve the nutritional quality of soy meal as animal feed. Following genetic transformation, the 2S protein from Brazil nuts constituted a significant fraction of the soybean protein. Since Brazil nuts are allergenic, *in vitro* tests, including RAST and immunoblots, were used to test extracts of the transgenic soybeans for expression of Brazil nut allergens. Sera from 8 out of 9 Brazil-nut-sensitive subjects reacted to transgenic soybean extract (see Figure 1), confirming that an immunologically functional Brazil nut allergen had indeed been transferred to the soybeans [8]. Although Brazil nut allergy is not common, if the allergens were widely dispersed in a commodity food, such as soybeans, exposure and presumably reactivity would increase.

Using RAST inhibition and immunoblotting, we assessed the allergenic potential of transgenic soybeans engineered to produce elevated levels of oleic acid [9]. Virtually identical inhibition of wild-type extract and an extract prepared from transgenic soybeans was demonstrated, as can be seen in Figure 2. Results of immunoblotting demonstrated that both the wild-type and transgenic soybeans contained approximately 30 proteins bands ranging in mo-lecular weight from 14 to 100 kD; the intensity of the bands appeared to be identical. Results suggest that changes in soy proteins resulting in increased oleic acid content did not alter soy allergen levels and thus do not pose increased risk of allergy to consumers.

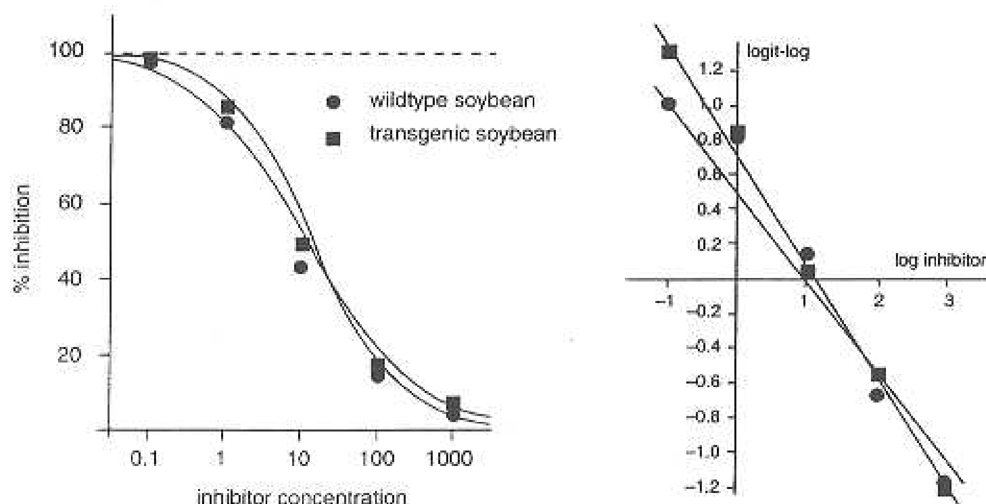

Figure 2. Inhibition curves and logit-log transformation of the wild-type soy RAST with transgenic and wild-type soy extracts. Statistical analysis showed that both the slopes and the y-axis intercepts are statistically identical indicating that the allergen contents and compositions of both soy extracts are identical.

Lastly, we investigated the allergenic activity of sulfur-rich corn proteins [10]. Two zein proteins, 10 kD and HSZ, were identified. Since efforts are still underway to increase the expression of these proteins in corn or the seeds of other cereal grains in order to enhance the sulfur content of sulfur-poor crops, it is important to assess their potential allergenicity. Sera from 42 individuals demonstrated by clinical history, skin test, or RAST and immunoblot to be corn reactive were tested for IgE antibody reactivity to these two zein corn proteins using SDS-PAGE/immunoblotting (see Figure 3). None of the sera from corn-reactive subjects demonstrated IgE reactivity against either the 10 kD or HSZ zein proteins, suggesting that products encoding these genes do not pose an increased risk of allergy to consumers.

Assessing Allergenicity in Genetically Engineered Foods Derived from Sources of Unknown Allergenicity

A much more difficult problem than the one outlined above is the evaluation of the allergenic potential of foods engineered using proteins from sources of undetermined allergenicity. Predicting potential allergenicity is a major challenge to the food industry since there is no single predictive assay for assessing the potential allergenicity of any proteins.

A preliminary step — evaluation of amino acid sequence homology — may be useful in predicting the allergenicity of a transgenic protein. When observing sequence homologies, particularly with regions containing IgE-binding epitopes, it is essential that one perform *in vitro* testing with RAST or ELISA to assess IgE reactivity. If no IgE antibody reactivity is detected, the second step should be to compare the physicochemical and biologic characteristics, including molecular size, stability, solubility, and isoelectric point of these proteins with major food allergens. Suspect proteins should be tested again to assess IgE reactivity. Clearly, these approaches may be extremely difficult, if not impossible, to establish if IgE reactivity is not known.

Screening Amino Acid Sequence Homologies with Known Allergens

Over 200 allergens have been identified, characterized, and sequenced; this information is available through public domain data bases (i.e., Gen Bank, PIR, EMBL, and Swiss Prot). S allergens from food and nonfood sources can cross-react, it is ical to compare amino acid sequences from all known aller — not just with food allergens — with those from the genetic engineered food. Based on the optimal peptide length for b ing B-cell epitopes (8–12 amino acids), an immunologically nificant sequence would require a match of at least eight co uous identical or similar amino acids. Confirmation of su sequence suggests that the transferred protein may be aller ic, and *in vitro* testing as described above should be employe further assessment of the allergenicity of the protein. Failu find a such a match strongly suggests that the introduced pro does not share linear epitopes with known allergens, but this not exclude the possibility that the transferred protein is an a gen itself.

Amino acid sequences are important. Yet, this approac limited since it does not take into account the importance of epitope interactions to the reactivity of protein molecules IgE antibodies, or the presence of discontinuous conformat al epitopes. Moreover, amino acid sequence similarity does necessarily mean similar IgE reactivity [11]. The degree of mology must also be considered. Recently, it was shown that tation of a single amino acid in the major peanut allergen Ara could abate, diminish, or enhance IgE binding, depending on substitution [12]. Thus, for a 10 residue region of a transg protein, an amino acid sequence identity of 90% with a kn allergen epitope does not prove a clinically significant cr reactivity based on the results with IgE binding epitopes.

Comparison of Physicochemical and Biological Characteristics

As stated above, food allergens generally share physicoch ical and biological characteristics, including molecular size, bility, solubility, and isoelectric point. Thus, it is beneficia compare these aspects in proteins of transgenic foods with kno food allergens. Of these traits, stability to digestive processes n be the most important when assessing potential allergenicity. P teins that are stable under the proteolytic and acidic conditi of the digestive tract are more likely than more labile protein reach the intestinal mucosa and stimulate an immune respon

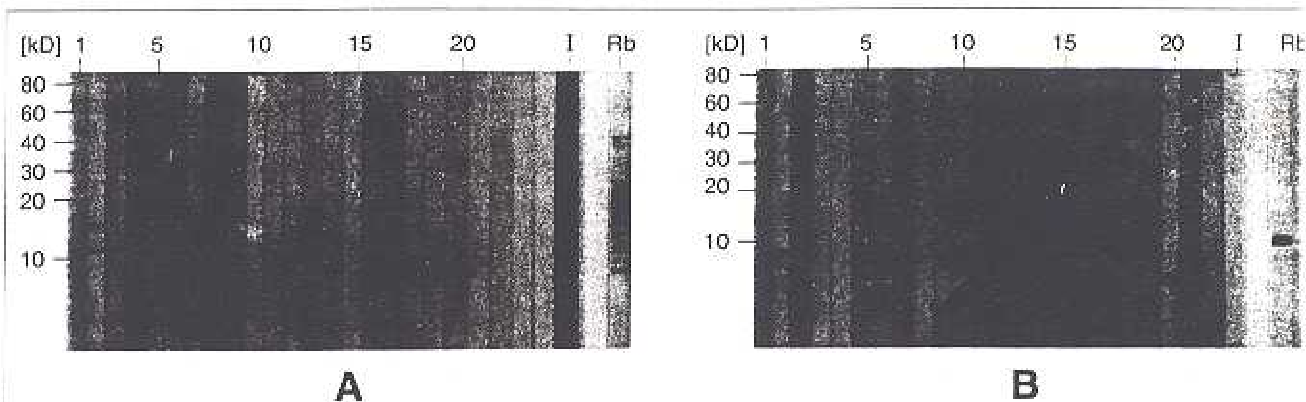

Figure 3. IgE antibody reactivity to the HSZ (A) and 10 kD zein (B) recombinant corn protein. Lanes 1–22: IgE reactivity of individual subject sera; I: protein stain with India ink; Rb: positive controls with HSZ and 10 kD zein-specific rabbit antisera, respectively.

Thus, rapid degradation of proteins expressed in genetically engineered foods reduces the likelihood that the protein is an allergen.

Recently, an *in vitro* model to assess the stability of food allergens to digestion was developed [13]. In this model, known food allergens and other common plant proteins were exposed to simulated gastric fluid for varying periods of time. Food allergens were stable under these conditions for periods up to 2 min — with some major allergens being stable for more than 1 hour. The few nonallergenic food proteins tested, on the other hand, were degraded within 30 seconds. It should be emphasized that stability of a transgenic protein in simulated gastric acid is not synonymous with allergenicity since some allergens are rapidly degraded by proteolytic enzymes. Furthermore, there needs to be more detailed studies on the enzyme stability of nonallergenic food proteins.

Lastly, the prevalence of the protein in a food should also be considered. In plant foods, many of the allergens are storage proteins present in large amounts. And in general many food allergens constitute a large proportion of total protein (1–80%) in offending foods [14]. Introduced proteins are usually expressed in plants in very low levels: from less than 0.01% to 0.4% [2]. It is tempting to speculate that the degree of exposure to allergens, i.e., the amount of an allergen in a food, is directly related to allergenic potential. However, the major allergen in codfish, Gad c 1, is not a predominant protein, while proteins that are major components of many foods, including myosin, tropomyosin, and actin from beef, pork, and chicken, have not been identified as major allergens.

Future Directions

Although this review has focused on problems arising from transferring known allergens or creating new allergens, bioengineering techniques also provide a unique opportunity to create hypoallergenic varieties of plants. Matsuda and coworkers [15] genetically engineered a hypoallergenic rice. A seed protein of approximately 16 kDa was identified as a major rice allergen. By introducing genes in the antisense orientation, the levels of expression of the allergenic protein were reduced compared to the wild type. The exciting study by Stanley and associates [12] suggests that altering IgE-binding epitopes of the peanut allergen, Ara h 2, could substantially reduce allergenicity of this ubiquitous food. Thus, biotechnology may be used to alter the allergenic potential of foods.

Summary

Recombinant techniques can be used to offer breeders, horticulturists, and farmers agricultural crops with improved qualities, including increased resistance to insects, disease, and herbicides. Since the genes governing the coding of proteins conferring these new traits are not part of the plant's original genome, there are concerns for the safety of these newly engineered varieties. A major concern is the allergenic potential of transferred proteins. Systematic strategies to assess potential allergenicity of these proteins have been proposed, but such approaches must be modified as more becomes known about the structural basis of allergenicity. The main problem is evaluating the allergenic potential of proteins not known to be allergenic. There is no definitive way to do this. Recent approaches include comparison of amino acid sequences of the transferred protein with those of known food and nonfood allergens, and comparison of chemical and physical characteristics of the transferred protein with known food allergens. *In vitro* assays using sera from hypersensitive individuals or placebo-controlled double-blind food challenges should be used to confirm the allergenicity of any transgenic foods prior to marketing.

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