

Review article

Risks of allergic reactions to biotech proteins in foods: perception and reality

In recent years, significant attention has been paid to the use of biotechnology to improve the quality and quantity of the food supply due in part to the projected growth in the world population, plus limited options available for increasing the amount of land under cultivation. Alterations in the food supply induced by classical breeding and selection methods typically involve the movement of large portions of genomic DNA between different plant varieties to obtain the desired trait. This is in contrast to techniques of genetic engineering which allows the selection and transfers specific genes from one species to another. The primary allergy risk to consumers from genetically modified crops may be placed into one of three categories. The first represents the highest risk to the allergic consumer is the transfer of known allergen or cross-reacting allergen into a food crop. The second category, representing an intermediate risk to the consumer, is the potential for replacing the endogenous allergenicity of a genetically-modified crop. The last category involves expression of novel proteins that may become allergens in man and generally represents a relatively low risk to the consumer, although this possibility has received attention of late. In order to mitigate the three categories of potential allergy risk associated with biotech crops, all genes introduced into food crops undergo a series of tests designed to determine if the biotech protein exhibits properties of known food allergens. The result of this risk assessment process to date is that no biotech proteins in foods have been documented to cause allergic reactions. These results indicate that the current assessment process is robust, although as science of allergy and allergens evolves, new information and new technology should help further the assessment process for potential allergenicity.

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Biotechnology has the potential to have a significant, positive impact on the food supply. However, there are also potential risks that must be addressed, including the possibility of allergic reactions to biotech proteins. In this article, we attempt to put into perspective the potential relative risk of allergic reactions to biotech proteins in foods by describing the prevalence of allergic reactions in the general population and the allergy assessment process that all biotech proteins are subjected to prior to their being allowed into the food supply. We also describe one of the potential benefits of biotechnology, the development of foods containing hypoallergenic forms of known allergens.

Biotechnology in the context of a changing food supply

In recent years, considerable attention has been paid to the use of biotechnology to improve the quantity and quality of the food supply. This interest is fueled, in part, by a growing world population that is expected to double by the year 2025, coupled with the realization that there are

limited options for increasing the amount of land under cultivation for the production of food crops without imposing undesirable environmental costs (1). In order to feed this growing population, crop yield must be increased, with some of the increase in yield due to the genetic engineering of foods. Genetically engineered food crops currently engender concerns about the potential for unintended health effects brought about by an altered food supply. There is nothing new in a changing food supply, however, inasmuch as the composition of the food supply has been changing for thousands of years as humans first cultivated wild grasses as sources of grain and bred domesticated animals for the production of meat and milk products as food sources in central Asia. During the last century alone plant and animal food sources changed through classical breeding and selection techniques. As a result of the use of these classical methods, poultry, in the mid-1940s one of the most expensive sources of meat, became one of the cheapest. In addition, hybrid grain varieties, developed during the so-called green revolution in the 1960s and 1970s, literally doubled

grain production, particularly in developing countries. These changes in no small part contributed to world stability and prosperity seen during the 1980s and 1990s.

Breeding and selection vs genetic engineering

Alterations in the food supply brought about by classical breeding and selection methods typically involved the movement of large portions of genomic DNA between different plant varieties in order to obtain the desired trait. This is in contrast to the techniques of genetic engineering or, as some call it, molecular breeding. The technology of genetic engineering allows the selection and transfer of a specific gene from one species to another. As this method can generally determine the gene that is transferred, genetic engineering is more selective as compared with other breeding techniques. Proponents of the use of genetic engineering technology to improve the food supply cite numerous realized and potential benefits to mankind including less expensive and healthier foods, hypoallergenic foods, the elimination of deficiency diseases, feeding a growing world population, reducing organic pesticide use, and reduction in loss of habitat. Critics of this technology raise concerns regarding the impact of genetically modified (GM) crops on the environment and consumers, including the potential risk of allergy from the new products.

Risks of food allergy

Food allergies occur in approximately 2–8% of the population with most food allergies associated with only eight foods or food groups (2–4). Furthermore, allergenic foods may contain up to 20 000 proteins, but typically only 10–20 of these may be allergenic. Thus, the chances of being exposed to an allergenic food protein or developing specific food allergies are low.

In spite of this, there are several misconceptions with regard to development of food-induced allergic reactions. For example, a consumer survey regarding the prevalence of adverse reactions to foods indicated that 30% of the people interviewed felt that they or some family members had an allergy to a food product (5). This survey also found that 22% of those interviewed avoided particular foods on the mere possibility that the food may contain an allergen. Clearly these figures are much higher than the actual prevalence of 2–8% mentioned previously.

Exposure requirements for the development of sensitization to a particular food are also not well understood by the public. An allergen is not a toxin in the classical sense of the term and thus does not elicit a reaction without the prior exposure (sensitization) of a susceptible individual. In fact, at least two, and probably more, exposures are required to induce sensitization to a particular food. Furthermore, development of an allergic reaction is a complex process involving an immune system that has a

genetic predisposition to propagation of an IgE antibody response (6). Thus, the risk of food allergy in the general population is considered to be quite low.

Genetically modified foods and allergy

The primary allergy risks to consumers from GM crops may be placed into one of three categories. The first category, representing the highest risk to the allergic consumer, is the transfer of a known allergen or a cross-reactive allergen into a food crop. For example, placing a gene encoding a known peanut allergen into corn would potentially make this GM crop unsafe to all peanut-allergic individuals. This actually occurred during attempts to produce a nutritionally enhanced soybean which expressed a Brazil nut protein. Based on the analysis of Steve Taylor's laboratory (7), the development of this transgenic soybean expressing a Brazil nut allergen was stopped.

The second category, representing an intermediate risk to the consumer, is the potential for increasing the endogenous allergenicity of a GM crop. The potential alteration of levels of endogenous allergenic proteins due to the transformation process is a possibility and could cause an increased concern for already allergic patients. However, several studies have explored this possibility and found no difference in the allergenic potential of biotech foods when compared directly with their nonbiotech counterparts (8, 9).

The last category, expression of novel proteins that may become allergens in man, generally represents a relatively low risk to the consumer, although this possibility has received attention in the press and lay literature. As the use of biotechnology increases and different proteins are expressed, such proteins may be derived from sources to which there is little, if any, human exposure. Thus, there is the potential that these proteins may become allergens and, therefore, a sound allergy-assessment strategy based on scientific principles is important to ensure the safety of the food supply.

A number of governmental agencies have addressed the issue of allergenicity and GM foods. Several of the European Union agencies, as well as individual governmental organizations within the member states, have been proactive in advocating a rigorous allergy assessment of GM crops. In the United States, such assessments have been the purview of the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Department of Agriculture (USDA). Furthermore, industry organizations such as the International Life Sciences Institute (ILSI), the Allergy and Immunology Institute (AII), and the International Food Biotechnology Council (IFBC), as well as nongovernmental and nonindustry international organizations such as the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO), have organized meetings of experts and advisory

panels to address this issue. The involvement and interest of these organizations have resulted in a robust allergy risk assessment process for products developed through biotechnology. This process was first published in 1996 (10) and has been refined and modified by international organizations such as FAO/WHO (11, 12) and the Codex Alimentarius Commission (13).

Allergy assessment of biotech proteins in foods

In order to mitigate the three categories of potential allergy risk associated with biotech crops, all genes introduced into food crops undergo a series of tests designed to determine if the biotech protein exhibits properties of known food allergens. All biotech proteins are assessed as to their source (allergenic or nonallergenic), any amino acid sequence similarity to known allergens, and their stability to digestion with proteases from the GI tract. For a detailed review of the allergy assessment process as it is applied to GM food crops, see Astwood et al. (14). As a result of the rigors of this risk assessment process, no biotech proteins in foods have been documented to cause allergic reactions. Indeed, even with a biotech protein (Cry9c) considered to have a medium likelihood of being an allergen, no protein-specific IgE could be detected in patients suspected of having adverse reactions to corn containing this protein, and a double-blind-placebo-controlled-food challenge of one patient was negative (15). These results indicate that the current assessment process is robust. However, the science of allergy and allergens is still evolving, and with new information, new technologies are being developed that will help to further assess the potential allergenicity of novel proteins and foods in the food supply.

One such improvement may be the development of animal models to predict allergenicity for humans. In recent years, several animal species, including rats, mice, pigs, and dogs, have been investigated for their usefulness in delineating the mechanisms involved in both the sensitization and elicitation phase of food allergic reactions (16–23). Each animal model studied a number of variables (24) such as route of sensitization (oral *vs* parenteral) and the use of adjuvants as well as the antigens used (whole food, crude food extracts, purified food allergens). All of these models have various advantages and disadvantages and are providing important information with regard to the mechanisms of food-allergic reactions. Unfortunately, there are no validated models currently available that are generally accepted for predicting the allergenic potential of specific proteins in human naïve subjects.

Recently, the Lehrer laboratory investigated the allergenicity of major food allergen extracts in different strains of mice (23, 25). Major food allergens, such as those found in peanuts, shrimp, walnuts, and cashews, stimulated significant IgE antibody responses. In con-

Table 1. Mouse *vs* man

Food extract tested	Mouse IgE antibody response	Allergenicity in man
Peanuts	++++	++++
Shrimp	++++	++++
Tree nuts	++++	+++
Rice	–	+
Beef	–	–
Chicken	–	–

trast, there was a lack of minimal IgE antibody production in mice immunized with nonallergenic foods such as rice, beef, and chicken (Table 1). The results are encouraging in that they suggest that mice may respond similarly as man in terms of elevated IgE antibody production to foods delivered through the oral route. Analysis of IgE antibody reactivity to two major food allergens (found in shrimp and peanuts) by immunoblotting demonstrated substantial IgE antibody reactivity to the same major allergens within these foods to which humans react (Fig. 1). Thus, the results with these animal models as well as others are encouraging and allow for a sense of optimism about the development of such an approach for testing novel proteins in the future.

Biotechnology and the potential for development of foods with reduced allergenicity

Biotechnology is being used in several ways to enhance the positive health effects of foods and to reduce or abolish possible negative effects. The potential severity of clinical allergic symptoms has led some scientists to explore methods to reduce the allergenicity of foods using genetic engineering methods. For example, genetic engineering can be used to reduce allergenicity by a variety of methods including post-transcriptional gene silencing, alteration of an allergen's secondary or tertiary structure, and modification of the primary amino acid sequence of genes encoding allergens.

Post-transcriptional gene silencing

Herman et al. (26) have successfully silenced a major allergen in soy using this technology. Transgene-induced gene silencing was used to prevent the accumulation of the Gly m Bd 30 K protein, a major soy allergen (27), in soybean seeds. Importantly, the Gly m Bd 30 K-silenced plants and their seeds lacked any compositional, developmental, structural, or ultrastructural phenotypic differences when compared with control plants. While this is a significant experimental breakthrough it must be viewed with some caution. There are numerous proteins that have been identified as allergens in soy (28) and it is unclear as to whether the suppression of only one gene will prove beneficial to soy allergic patients.

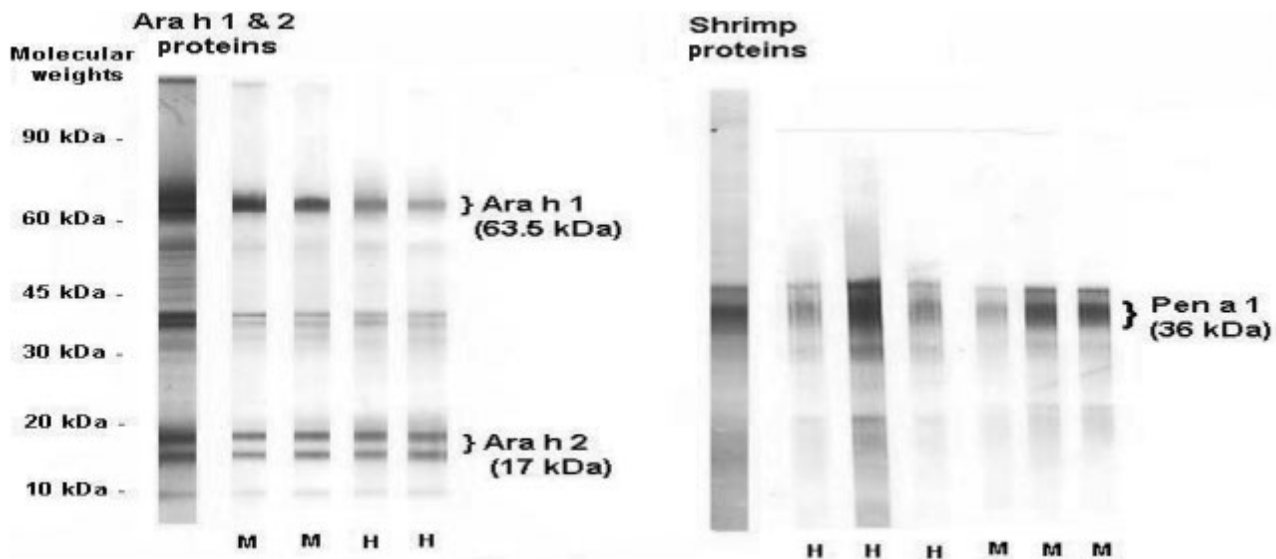


Figure 1. Comparison of Murine (M) with human (H) IgE reactivity to peanut Ara h 1 and Ara h 2 and shrimp Pen a 1.

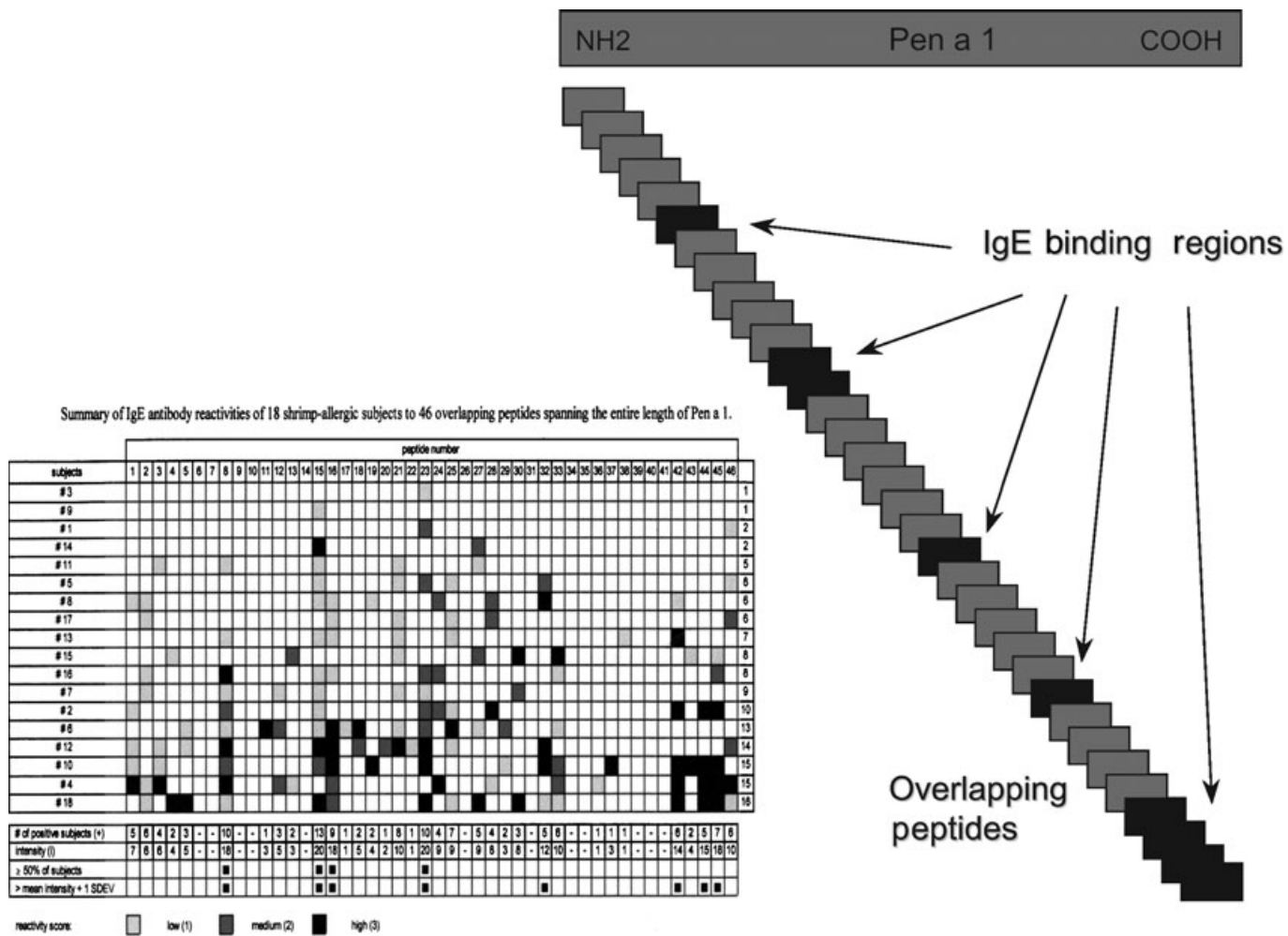


Figure 2. Epitope mapping of Pen a 1.

Alteration of allergen secondary or tertiary structure

The biological activity of thioredoxins, coupled with the observation that many food allergens are proteins containing intramolecular disulfide bonds that may be important to their allergenicity (29), raises the possibility that thioredoxin could be used to reduce the allergenic potential of some foods. To test this concept, Buchanan and colleagues exposed either the purified allergens from wheat and milk or an extract from these food sources containing the allergens to thioredoxin purified from *E. coli* and then performed skin tests and monitored gastrointestinal symptoms in a sensitized-dog model (30, 31). Allergens that had their disulfide bonds reduced by thioredoxin showed greatly reduced skin reactions and gastrointestinal symptoms. These results provided a critical proof-of-concept for this approach prior to constructing transgenic wheat lines that overproduce thioredoxin.

Alteration of allergen primary amino acid sequence

Studies to reduce the allergenicity of major peanut, soy, and shrimp allergens by amino acid sequence alteration have been described (32–40). It has been shown through a number of studies that the major shrimp allergen and, indeed, a major allergen in most crustacea, arachnids, and insects is the muscle protein tropomyosin. The portions of shrimp tropomyosin that bind IgE, the allergenic epitopes, can vary in size from about eight to 15 amino acids. Using synthetic overlapping peptides and the SPOT system of analysis, five major IgE-binding regions containing eight major IgE-binding epitopes present in shrimp tropomyosin were identified (Fig. 2). Analysis of the effects of amino acid substitution of these regions through the use of synthetic peptides and the SPOT system identified mutated epitopes that profoundly affect IgE antibody binding. As little as one amino acid substitution may result in no change, enhancement, reduction, or complete abolishment of IgE antibody binding. Thus, such changes can produce an allergenically inactive molecule. If the T-cell epitope sites are preserved, such molecules should still be active in desensitization of allergic subjects while reducing the risk

of an allergic reaction during treatment. Furthermore, incorporation of these mutations into the genome of the organism or plant may lead to the development of a product with reduced or abolished allergenic activity. This is one approach being taken to develop altered plant and animal sources of foods such as peanut, soy, or shrimp with reduced or abolished allergenic activity.

Concluding remarks

Food allergy is an often misunderstood, rare disease that has both a genetic and environmental component that contributes to its development. As no known cure is available for those afflicted with food allergies, disease management is achieved by avoidance of the offending food. As a result, significant weight in the assessment of a biotech protein is given to the need for exposure prevention, which, in the context of safety assessment, means reducing the likelihood of transferring offending allergens from one food to another. The allergy assessment testing strategy is a tiered, hazard identification approach that utilizes currently available scientific data regarding allergens and the allergic response. This approach has worked to ensure the safety of the current wave of pest-resistant and herbicide-tolerant crops as shown by the lack of any substantiated health-related claims linked to biotech crops. However, the science of allergy and allergens is still evolving, and the development of new information and new technologies will help to further assess the allergenicity of novel proteins and foods in the food supply. Finally, the use of biotechnology to provide healthier food, including food with reduced or abolished allergenicity, is on the horizon and should help improve the quality of the food supply.

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