Potential Health Risks of Genetically Modified Organisms: How Can Allergens be Assessed and Minimized?

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Allergies to foods are a significant public health concern throughout the world. Nearly 2 percent of adults and 4-6 percent of children suffer from food allergies, which are defined as an adverse immunologically mediated reaction to antigenic molecules present in foods. The allergic response is mediated by the interaction of cell-bound immunoglobulin E (IgE), a class of antibody molecules uniquely involved in allergic reactions with allergen (Sampson and Metcalfe 1991; Metcalfe, Sampson, and Simon 1996). Antigenic molecules, or allergens, typically are proteins that stimulate allergen-specific IgE production in certain individuals through as yet undetermined mechanisms (Lehrer, Horner, and Reese 1996; Taylor and Lehrer 1996). Inheritance and exposure to allergens are two factors that contribute to the development of allergy. The pathophysiological mechanisms of food allergies are distinct from other food intolerances such as gluten sensitive enteropathy (celiac disease) that are due to nontoxic, nonimmune reactions to foods even though symptoms may resemble those of "true" food allergies. Food allergy symptoms can range from mild discomfort to life-threatening anaphylactic shock (Sampson and Metcalfe 1991; Metcalfe, Sampson, and Simon 1996).

More than 90 percent of the allergic reactions observed in children and adults can be attributed to exposure to eight foods or food groups. These include eggs, fish, shellfish, milk, peanuts, soy beans, tree nuts, and wheat (Taylor and Lehrer 1996). Virtually all allergens are proteins; yet of the enormous numbers of proteins occurring in foods, only a very few are allergenic and only in certain people. Most characterized food allergens, with some exceptions, are stable to digestion and processing, and many of the major allergens are generally proteins that are present in large amounts in allergenic foods. The primary structure of food allergens has been determined by molecular cloning and sequencing (Taylor and Lehrer 1996; Bush and Hefle 1996; Lehrer and others 1997; Reese and Lehrer 1998).

Usually, food allergy develops as follows. An allergen or an immunologically active fragment of that particular allergen crosses from the lumen of the gut through the mucosal membrane barrier; this molecule or its fragment can stimulate different types of lymphocytes that ultimately result in the production of allergen-specific IgE antibodies. These IgE antibodies also have the unique ability to bind to surface receptors on mast cells and basophils; upon a second or subsequent exposure, allergen may bridge cell-bound IgE antibodies. The cross-linking of cell-bound IgE antibodies will lead to the release of both preformed mediators, such as histamine and newly synthesized mediators such as protaglandins, that cause smooth muscle contraction, vasodilation, bronchial constriction, and cell infiltration, which in effect cause symptoms of allergic reactions (Sampson and Metcalfe 1991).

Food Allergens

Generally, food allergens seem to share several common properties; they are proteins or glycoproteins with an acidic isoelectric point (pI), and

usually are in the molecular weight range of 10,000 to 80,000 daltons. Most food allergens are fairly resistant to industrial processing, heating, and cooking, as well as showing some resistance to the digestive enzymes of the gut (Lehrer, Horner, and Reese 1996). These properties may aid in the allergenicity of those molecules. These properties are not, however, necessarily unique for food allergens since they can also occur in nonallergenic molecules (Lehrer, Horner, and Reese 1996; Lehrer and others 1997). Substantial in vitro cross-reactivity (sharing similar immunochemical structures) can occur among foods and between foods and other substances (Reese and Lehrer 1998). This can occur within closely related food groups such as crustacea and legumes; however, such in vitro cross-reactivity is not always reflected by clinical cross-sensitivity (Metcalfe, Sampson, and Simon 1996). In addition, foods and seemingly unrelated substances have been shown to cross-react. For example, grass and tree pollens as well as latex allergens cross-react with a variety of fruits and vegetables, oysters have been shown to have common allergens with crustacea and insects and shrimp share allergens with dust mite and cockroaches. The precise nature of cross-reactivity of such unrelated substances is not entirely known but may be due to the presence of common structural or functional proteins (Lehrer and others 1997; Reese and Lehrer 1998). Food allergens that have been characterized are summarized in Table 1. Because many food proteins have been studied for reasons other than their allergenicity (that is, because they are important storage, structural, and functional proteins), it is often possible to identify a food allergen by searching protein databases using only a small part of its entire amino acid sequence (Reese and Lehrer 1998).

Genetically Modified Crops: Allergenicity Risk Assessment

Modern biotechnology provides methods for the identification and selection of genes encoding for specific proteins. A gene from any source (for example, microorganism, plant, or animal) that confers a specific trait can be selectively and precisely introduced or transferred into the genome of another organism where the expression of the transferred gene will confer that desired trait on the host organism. This type of genetic engineering has been used to introduce genes into various microorganisms and plants that are sources of foods and food components. Introduced traits include insect and virus resistance, herbicide tolerance, and changes in composition or nutritional content. Typically the amount of protein expressed by the introduced gene is small and, in some cases, inactivation of a native gene that results in the absence of a specific protein yields the desired trait (for example, the tomato genetically engineered to delay ripening). This technology has also been used to reduce the expression of a major allergen found in rice (Matsuda and others 1993).

Genetically modified crops, now grown on some 40 million hectares around the world, are changing modern agricultural methods. Supporters of this approach believe that genetic engineering is crucial in developing healthy, productive crops that are essential to feed the world's growing populations. Thus, although these methods are being used primarily in the industrial countries, this approach is also important in developing countries. In contrast, critics of GM foods raised concerns because of the unusual methods used to breed these crops; some fear that the genetic variants produced could introduce foreign substances into the food supply with unanticipated negative effects on human health and the environment (Schmidt 2000). A major concern is that a protein encoded by an introduced gene may be allergenic and cause allergic reactions in exposed populations.

Until recently, genetically improved crops that were introduced in a number of industrial countries were favorably received by both domestic and international regulatory bodies; a number of new crop varieties have been successfully marketed to farmers in the United States, China, Argentina, Canada, Australia, and Mexico, amongst others. However, public attitudes toward GM crops have recently diminished, particularly in Europe (Schmidt 2000).

When addressing the potential health risks of GM crops in developing countries, two questions must be considered. First, what are the risks and how can these risks be assessed and minimized? Second, how do these risks relate to benefits for

Table 1 Identified and characterized major food allergens (Lehrer and others 1997, modified). [1]References for partial (P) or complete (C) sequence data

Allergen Source	*Allergens (Systematic and original names)*	*MW (kDa)*	*Sequence Data*	*References*[1]
Gadus callaria (cod)	Gad c 1; allergen M	12	C	Elsayed and Bennich 1975
Gallus domesticus (chicken)	Gal d 1; ovomucoid	28	C	Hoffman 1983
	Gal d 2; ovalbumin	44	C	Langeland 1983b
	Gal d 3; conalbumin (Ag22)	78	C	Williams et al. 1982
	Gal d 4; lysozyme	14	C	Blake et al. 1965
Penaeus aztecus (brown shrimp)	Pen a 1; tropomyosin	36	P	Daul et al. 1993, 1994
Penaeus indicus (indian shrimp)	Pen i 1; tropomyosin	34	P	Shanti et al. 1993
Metapenaeus enis (greasyback shrimp)	Met e 1; tropomyosin	34	C	Leung et al. 1994
Brassica juncea (oriental mustard)	Bra j 1; 25S albumin	14	C	Monslave et al. 1993
Hordeum vulgare (barley)	Hor v 1; BMAI-1	15	C	Mena et al. 1993
Sinapis alba (yellow mustard)	Sin a 1; 25S albumin	14	C	Menendez-Arias et al. 1988
Arachis hypogaea (peanut)	Ara h 1	63.5	C	Burks et al. 1995a,1995b, 1995c
	Ara h 2	17.5	C	Stanley et al. 1997
Malus domestica (apple)	Mal d 1	17.7	C	Vanek-Krebitz et al. 1995
Apium graveolens (celery)	Api g 1	16.2	C	Breiteneder et al. 1995

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the exposed populations? In most industrial countries, where a number of genetically improved crops have been marketed, the public concerns of allergy may be somewhat greater than those of developing countries with emerging economies, where allergy is a less pressing issue among their public health and nutrition concerns. Thus allergy, a disease more frequent among the middle and upper classes of industrial countries, may be considered less of a risk in developing countries as compared to industrial countries.

Theoretically, there are two ways in which genetic modification may alter the allergenicity of a food. First, the level of endogenous proteins within a particular crop may be altered by genetic manipulation, potentially raising the level of endogenous allergens. Second, the expression of a new gene in this crop could introduce new allergens normally not present in this particular crop. Thus, there can be an effect on known allergens or unknown allergens. If the endogenous proteins or the newly introduced protein are from known sources of allergens, then assessing the allergens within the GI food is relatively straightforward. However, a more difficult issue is if the allergenicity of the source of the protein is un-

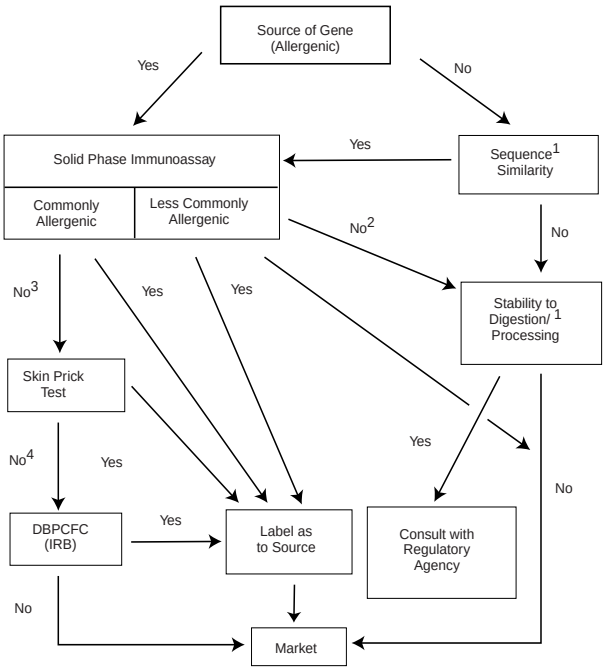
known; this generally relates only to new proteins being introduced into GM foods from sources that have ordinarily not been used as human food. The dilemma is that there is no available body of knowledge about the allergenicity of these proteins, and thus one would have to rely on other criteria with which to assess their potential activity.

The ILSI Allergy and Immunology Institute and the International Food Biotechnology Council convened an expert panel of scientists to develop scientific approaches to assess the allergic potential of foods derived from GM crop plants. This initiative resulted in the development of a published project report (Metcalfe and others 1996). This report addressed the cell biology, symptoms, and treatment of food allergy; developed a catalog of allergenic foods; and characterized major food allergens from the perspective of the plants and methods used to genetically modify food crops. This information served as the background for the development of a decision tree for assessing the allergic potential of foods derived from genetically engineered plants (Figure 1).

Eight commonly allergenic foods and more than 160 less commonly allergenic foods were identified. Based on this information, the report concluded that food biotechnologists should avoid the transfer of known food allergens. Genes transferred from sources known to be allergenic should be assumed to encode for an allergen, until proven otherwise. In addition, the allergenic potential of all introduced proteins should be assessed. For genetically improved foods entering the marketplace, consumers should be informed by appropriate labeling that the food contains known or suspected allergens (Metcalfe and others 1996).

The safety assessment decision tree (Figure 1) begins with the characterization of the source of the introduced gene. Is it from a commonly allergenic or less commonly allergenic source or does the source have no history of allergenicity? If there is no history of allergenicity associated with the gene source, its protein product should be subjected to amino acid sequence analysis. The sequence should be compared with those of the more than 180 known allergens that have been deposited into various electronic databases (for example, GenBank, EMBL, SwissProt, PIR). Available software can evaluate for amino acid sequence homologies, structural similarities, and epitope mapping based on eight contiguous amino acids (that is, the suggested minimum size of allergenic epitopes). If this evaluation fails to provide evidence suggesting allergenic potential, the protein should then be subject to physical/chemical testing to establish its stability to digestion and processing. Proteins that are labile to digestion are unlikely to be allergenic. A food containing a protein for which there is no concern based on amino acid sequence or on chemi-

Figure 1 Decision tree for assessment of the allergenic potential of foods derived from genetically engineered food crops.



Notes:

1. It is recommended that an assessment for amino acid sequence similarity to all known allergens and an assessment of stability to digestion be performed for all gene products.
2. Solid phase immunoassay tests depend on availability of sera. Ideally, 14 sera should be used. However, if less than 5 sera are used, then proceed to stability box if results are negative and consult with the appropriate regulatory agency.
3. In the case of equivocal results or suspected false positives, proceed to skin prick tests.
4. DBPCFCs are performed on food products in which there is no evidence of allergenicity based upon solid phase immunoassays and skin prick tests. To assure lack of allergenicity, DBPCFCs should be performed following IRB approval.

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cal analysis would not be considered to have allergenic potential (Metcalfe and others 1996).

If the protein originates from a known allergenic source or its amino acid sequence analysis raises concern about the allergenic potential of the molecule, the protein is then evaluated to determine whether it is recognized by serum from individuals with known food allergies (Lehrer, Horner, and Reese 1996; Metcalfe and others 1996; Lehrer and Reese 1997a). Standard statistical methods can be used to estimate the number of sera samples that need to be tested to have a high probability (95.5-99.9 percent) of detecting both major and minor allergens. Equivocal results would necessitate conducting stability testing of the protein, whereas negative results would indicate that the allergenic potential of the protein is negligible. If the protein product of an introduced gene exhibits similarities to known allergens and/or yields positive results in serological analysis, the appropriate regulatory authority should be consulted to determine if and what further testing might be performed (Metcalfe and others 1996).

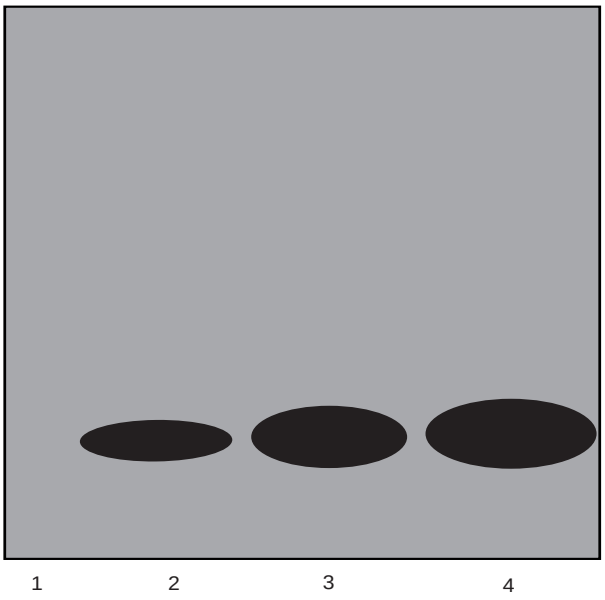
Genetically modified foods containing those proteins that test positive in the serologic analysis should be labeled as to the source of the protein. In addition, for proteins considered to be commonly allergenic based on the serological analysis, confirmatory skin prick testing is recommended. If these tests are positive, double-blind placebo-controlled food challenge testing should be conducted in accord with Institutional Review Board-approved protocols for the use of human subjects. Foods containing proteins confirmed as allergenic in the skin prick and/or food challenge studies could be brought to market with appropriate labeling, although foods confirmed to be allergenic by challenge testing would likely have only a very limited place in the market (Metcalfe and others 1996).

The major challenge for the food industry is testing the source of the gene from which there is no history of allergenic activity, since there is theoretically no known sera available from allergic subjects to test the product (Lehrer, Horner, and Reese 1996; Metcalfe and others 1996; Lehrer and Reese 1997a; 1998). The recommended approach is to compare the amino acid sequence of the protein with that of known allergens as described previously. Any sequence similarity with a particular allergen suggests the sera can be used to screen the product by immunochemical procedures described earlier. If there is no amino acid sequence homology, the stability of the protein to enzymatic digestion and processing can be assessed. If the molecule is easily digested or unstable then there should not be a problem with marketing the product. If, however, the molecule is stable to digestion and processing, then one would need to consult with regulatory authorities (Lehrer, Horner, and Reese 1996; Metcalfe and others 1996; Lehrer and Reese 1997a; 1998).

An actual case study that considers the introduction of a gene for a known allergen in a GM crop is the expression of a Brazil nut protein in soybean (Nordlee and others 1996). Because soybeans are deficient in essential sulfur-containing amino acids such as methionine and Brazil nuts are rich in this substance, food biotechnologists introduced a gene encoding a Brazil nut methionine-rich seed storage protein into soybean. Brazil nuts are known to be allergenic, however, raising concern whether the product of the transferred gene would increase the allergenic potential of the soybean. Because the protein is from a known allergenic source, serological evaluation of the protein was performed. In this case, pooled serum from nine Brazil nut-sensitive individuals recognized the protein, and eight of the nine sera bound to the protein in an immunoblotting assay (Figure 2). Skin prick tests with three of these individuals confirmed the presence of the allergen (Nordlee and others 1996). Based on these findings, development of this product was discontinued.

Another case study, in which allergenicity was not altered, is the high oleic acid soybeans developed by genetic modification. Soybeans were genetically engineered to enhance their oleic acid content, a property considered to produce healthier soybean oil. This genetic modification elevated the levels of several proteins. When the allergen content of the transgenic soybean was compared to the wild-type parental strain, there appeared to be no significant differences in activity based on RAST inhibition assays (Figure 3) as well as immunoblotting methods (Lehrer and Reese 1997b). Thus, qualitatively and quantitatively, the transgenic strain appeared to be allergenically the same as the parental wild-type (Lehrer and Reese 1997b). This indicates that IgE

Figure 2 Reactivity of Brazil nut allergenic serum to a 2 S albumin Brazil nut allergen



Notes: Lane 1: nontransgenic soybean; lane 2: transgenic soybean; lane 3: Brazil nut extract; lane 4: 9 kD 2S albumin Brazil nut allergen.
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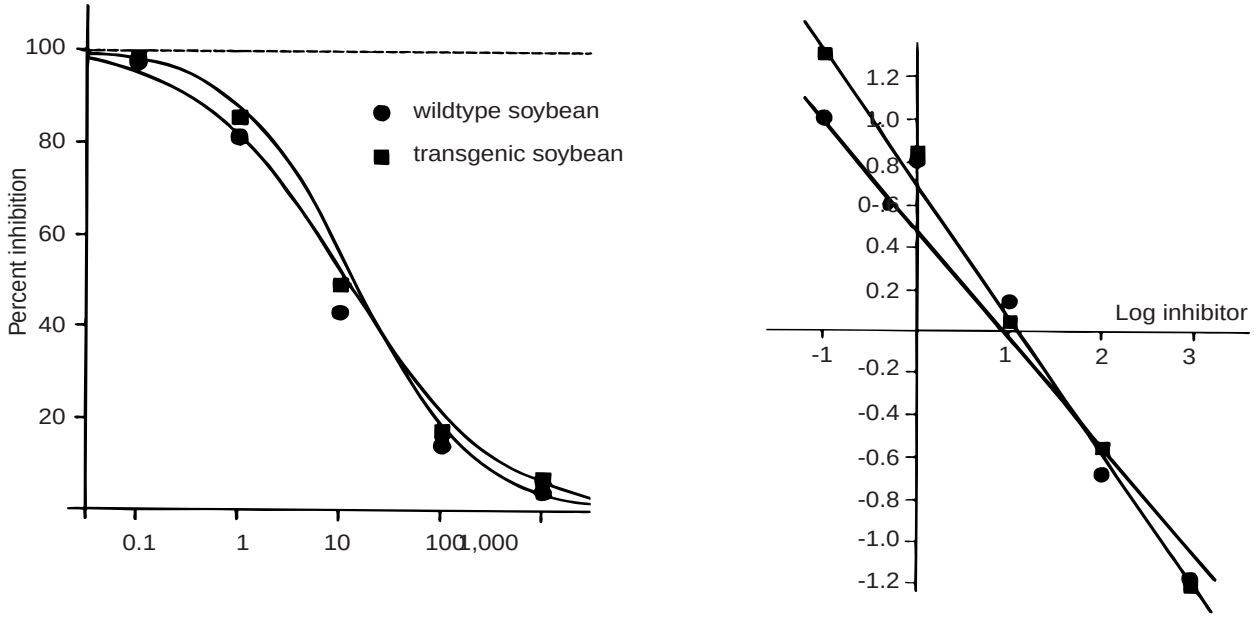
antibody-based assays used to test products developed from sources of known allergens can document no substantial change in the allergenic content. Thus, the probability that an introduced protein will be allergenic is low, and definitive methods are available to detect known allergens.

Conclusion

The assessment of the allergenicity of proteins from unknown allergen sources continues to be a challenge to the food industry. All evidence suggests there is no cause for concern about allergenic potential for proteins introduced into foods from sources with no history of allergenicity, that have no amino acid sequence similarities to known food allergens, that are rapidly digested, and that are expressed at low levels relative to the expression of major allergens. The recommended approach by amino acid sequence comparison and enzymatic digestion resistance is based on current technology available. Future efforts must be directed at refining this technology. This can be achieved through (a) continued allergen identification and amino acid sequence characterization to increase the number of aller-

Figure 3 Inhibition curves and logit-log transformation of the wild-type soy RAST with transgenic and wild-type soy extracts



Note: 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.
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genic sequences in the data bank; (b) identification of the amino acid sequence properties that define allergenic epitopes to develop more precise sequence screening criteria; and (c) development of an animal model that can recognize food allergens in a manner similar to that which occurs in human disease. In spite of the fact that the technology thus far used to assess the allergenicity of GM foods can be improved, it still serves us very well in identifying potentially allergenic products that may be developed. Thus, it is possible to identify potential risks for allergenicity and minimize their effect on exposed populations.

Last but not least, the risk-to-benefit ratio of these new technologies must be considered. A number of serological assays are being used to reduce the risk as stated above. The benefits derived from GM crops must be considered against these risks, which may vary from country to country. Allergy is a high priority among the middle and upper classes of industrial countries where any added risk in an already well-fed population may be a concern. However, in countries with emerging economies, where allergy is a lower priority than nutrition, the increased productivity benefits of GM crops may far outweigh any potential risk of allergic reactions.

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