Annu. Rev. Pharmacol. Toxicol. 2002. 42:99–112
Copyright © 2002 by Annual Reviews. All rights reserved

PROTEIN ALLERGENICITY ASSESSMENT OF FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY

Steve L. Taylor
University of Nebraska, Food Allergy Research and Resource Program, Lincoln, Nebraska 68583-0919; e-mail: staylor2@unl.edu

Key Words allergy, food, risk assessment, sequence homology

■ **Abstract** Foods produced through agricultural biotechnology are reaching the consumer marketplace. These novel foods should be assessed for their safety, including their potential allergenicity. Agricultural biotechnology involves the introduction of novel proteins into the modified foods, and proteins can be allergenic. The potential allergenicity of the introduced proteins can be evaluated by focusing on the source of the gene, the homology of the newly introduced protein to known allergens, the reactivity of the novel protein with IgE antibodies from the serum of individuals with known allergies to the source of the transferred DNA or to materials that are broadly related to the source of the transferred DNA, the resistance of the novel protein to pepsin, and the immunoreactivity of the novel protein in appropriate animal models. Additional factors, such as the level of expression of the novel protein in the modified food and expression in the edible portion of the food, may also yield valuable insights. Applying such criteria provides a reasonable approach to determining whether or not the novel protein is likely to become an allergen.

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY

Agricultural biotechnology has tremendous implications for all of agriculture and for the public. From the agricultural production perspective, agricultural biotechnology will likely be a major revolution in agriculture worldwide, although considerable resistance to the implementation of this new technology is certainly evident, especially in some parts of the world. From a consumer perspective, agricultural biotechnology holds great promise for improved quality characteristics, improved nutritional and health attributes, resistance to spoilage, and even reduced levels of allergens in foods (1).

The products of agricultural biotechnology are already appearing in the American and worldwide marketplace. Insect-resistant corn and herbicide-tolerant soybeans are now planted on 30% to 50% of the total acreage planted with these crops

in North America. As a result, food products containing ingredients derived from genetically modified corn and soybeans are quite common in North America. The introduction of genetically modified animal products has not yet occurred. However, a genetically modified salmon with enhanced growth characteristics is poised to enter the marketplace if regulatory approvals can be obtained.

Only a small number of crops with a limited number of traits have thus far been commercially developed through agricultural biotechnology (2). The crops include corn, potatoes, canola, soybeans, and cotton with either improved insect resistance or enhanced herbicide tolerance. Virus-resistant squash and papaya have also been introduced into the market. All of the existing traits in the current generation of genetically modified crops provide primarily agronomic benefits, although indirect benefits to consumers may also accrue (3). However, many more traits can potentially be developed in these and other crops in the future, including many traits that have direct consumer benefits. In the coming years, the potential exists for dozens of new products developed through agricultural biotechnology to enter the marketplace; these include crops that protect themselves from diseases and pests; crops that prosper under adverse conditions, such as heat, cold, and drought; and crops that look better, taste better, and provide better nutrition. For example, much publicity has surrounded the recent development of the so-called golden rice with enhanced levels of vitamin A (4), although golden rice is not yet commercially available and has not yet been through the necessary safety assessment process.

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

SAFETY OF FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY

Governmental regulatory agencies in most developed countries require a mandatory safety assessment and consultation with government regulators for all foods produced through agricultural biotechnology before allowing commercial sale. The genetically modified crops currently on the market have been thoroughly assessed for their safety under approaches recommended by the World Health Organization (WHO), the Food & Agriculture Organization of the United Nations (FAO) (5, 6), and other worldwide organizations. The general conclusion of these consultations is that the products of plant biotechnology are not inherently less safe than those developed by traditional breeding (7). Another critical conclusion is that the food safety considerations are basically of the same nature as those arising from the products of conventional breeding; therefore, traditional approaches to safety assessment are appropriate to use in the assessment of genetically modified foods. The accepted standard for genetically modified crops is identical to that expressed in the U.S. food laws for all food products—a reasonable certainty that no harm will result from intended uses under anticipated conditions of consumption.

The safety assessment of crops produced through agricultural biotechnology has often focused on the concept of substantial equivalence (5). In the current generation of genetically modified crops, only one or a few genes and their gene products have been inserted into the new variety. Under the concept of substantial

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

equivalence, the safety assessment is focused on those genes and their products that are introduced into the novel variety. The assumption is that, in most cases, the remainder of the crop genome and its gene products would be identical to that found in the parent variety and thus would be as safe as the traditional counterpart. Since ingested DNA is considered highly digestible and safe regardless of its source (8), the safety assessment process is typically focused on the proteins expressed from the introduced novel genes, and includes an assessment of the potential allergenicity of these novel proteins. Additionally, if the host plant is known to contain any antinutritional, toxic, or allergenic components, the novel plant would be tested to determine if the concentration of those components had been altered during the process of creating the new variety. The current generation of genetically modified plants that are being marketed would be considered as substantially equivalent to their traditional counterparts with the exception of the novel gene(s) and gene product(s) or protein(s).

In the future, the development of novel plants that are not considered to be substantially equivalent to their traditional counterpart is anticipated. This will occur because a larger number of genes have been introduced or the nutritional content has been altered in some significant manner. In such situations, a more thorough safety evaluation will be necessary. However, no such crops have yet been commercially developed, so the exact nature of the safety assessment that will be required by various government regulatory agencies around the world is not yet known.

In the safety assessment of foods produced through agricultural biotechnology, one of the key issues is the assessment of the potential allergenicity of the novel proteins introduced into these foods. The remainder of this review focuses on approaches to the assessment of the allergenicity of these new food products.

ASSESSMENT OF THE ALLERGENICITY OF FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY

The potential allergenicity of the newly introduced proteins must be assessed in all foods produced through agricultural biotechnology. Virtually all allergens are proteins. However, only a few of the many naturally occurring proteins found in foods are allergenic under typical circumstances of exposure. So, on a probabilistic basis alone, the likelihood that any specific introduced novel protein will become a new food allergen is rather small. However, the possibility must be addressed as part of the overall safety assessment process. Unfortunately, no single test is available that is fully predictive of the potential allergenicity of any specific novel protein. Thus, the current recommended strategy involves the use of a decision-tree approach employing several different tests, under the assumption that the overall predictability of the allergenicity of a novel protein will be improved by this combination of tests.

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

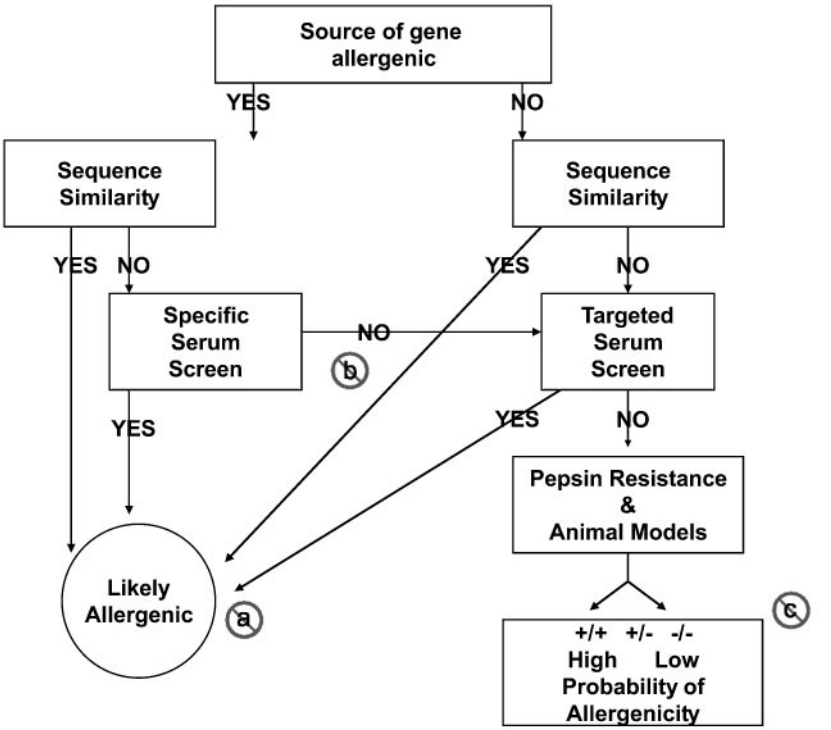
In 1996, a task force of the International Food Biotechnology Council (IFBC) and the Allergy & Immunology Institute of the International Life Sciences Institute (ILSI) developed the first decision-tree approach for the assessment of the potential allergenicity of plants produced through agricultural biotechnology (9). This approach focused on evaluating the source of the gene, the sequence homology of the newly introduced protein to known allergens, the immunoreactivity of the novel protein with serum IgE from individuals with known allergies to the source of the transferred DNA, and various physicochemical properties of the newly introduced protein such as heat stability and digestive stability. The genetically modified foods currently on the market were assessed for allergenicity using this recommended approach. While the application of this approach provided reasonable assurance that the newly introduced protein was unlikely to become an allergen, this decision-tree approach has been subjected to some criticism (10).

In January 2001, the FAO and WHO convened an expert consultation that developed a more rigorous approach to the assessment of the allergenicity of foods produced through agricultural biotechnology (11). This new decision tree is depicted in Figure 1. The new decision tree relies upon some of the same elements as the IFBC-ILSI decision tree, including the source of the gene, the sequence homology of the newly introduced protein to known allergens, the immunoreactivity of the novel protein with serum IgE from individuals with known allergies to the source of the transferred DNA, and resistance of the novel protein to digestion with pepsin. Additional criteria included in the new decision tree were the immunoreactivity of the novel protein with serum IgE from individuals with known allergies to species that are broadly related to the source of the transferred DNA and the immunogenicity of the novel protein in appropriate animal models. The additional rigor involved in the new decision tree will we hope inspire greater confidence in the accuracy of the assessments of potential allergenicity. Certainly, further modification of the new decision tree should be considered as science knowledge allows more accurate predictions regarding the potential allergenicity of novel proteins in humans. This review focuses on the scientific basis for the various tests advocated in the new FAO/WHO decision tree. The review also considers the importance of an additional criterion: the level of expression of the novel protein in the edible portion of the new plant variety.

Source of the Novel Gene

The FAO/WHO decision tree focuses first on the source of DNA to be introduced into the host organism. If the gene source is known to be allergenic, then the allergenicity of the gene product must be determined. This assessment would be conducted for DNA sources that are primarily environmental allergens, e.g., pollens, as well as for food allergens. Some known food allergens are crossreactive with pollen allergens (12). If the gene source is known to be allergenic, then the potential allergenicity of the novel gene product can be determined with a

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.



a Any positive results obtained from sequence homology comparisons to the sequences of known allergens in existing allergen databases or from serum screening protocols indicate that the expressed protein is likely allergenic.

b The degree of confidence in negative results obtained in the specific serum screen is enhanced by the examination of larger numbers of individual sera. Conducting the specific serum screen with small numbers of individual sera when larger numbers of such sera are readily available should be discouraged.

c When positive results are obtained in both the pepsin resistance and animal model protocols, the expressed protein has a high probability to become an allergen. When negative results are obtained in both protocols, the expressed protein is unlikely to become an allergen. When different results are obtained in the pepsin resistance and animal model protocols, the probability of allergenicity is intermediate, although rational explanations may be possible in some situations.

Figure 1 The FAO/WHO decision tree approach to the assessment of the allergenicity of novel proteins in genetically modified foods.

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

reasonable degree of certainty using the specific serum screening test outlined below that employs blood serum from human subjects known to be allergic to the source of the gene. The assumption must be made that the source gene in such circumstances encodes an allergen unless data are generated to disprove that assumption. Obviously, the greatest concerns are raised when the gene is obtained from a commonly allergenic source. Commonly allergenic foods include peanuts, soybeans, tree nuts, and wheat from the plant kingdom and milk, eggs, fish, and crustacea from the animal kingdom (13). These eight foods are thought to account for more than 90% of all food allergies on a worldwide basis (13). In addition to these foods or food groups, more than 160 foods and food-related substances have been associated with allergic reactions in individuals on at least some occasions (14).

If the gene is obtained from a source with no history of allergenicity, then a specific serum screening test is obviously not possible. In many cases in agricultural biotechnology, the gene is obtained from a source with no history of allergenicity.

Sequence Homology to Known Allergens

The amino acid sequences of many food and environmental allergens are known (9, 15). A comparison of the amino acid sequence homology of the novel protein to the amino acid sequences of known allergens is a useful initial approach in the determination of allergenic potential regardless of the source of the gene (9, 11). If sufficient homology exists, then suspicions would be raised regarding the possibility that the novel protein might crossreact with the known allergen and provoke symptoms when ingested by individuals with that particular allergy. The current criteria used to determine significant sequence similarity as proposed in the FAO/WHO strategy is a match of at least six contiguous, identical amino acids or an overall homology in excess of 35% (11).

In IgE-mediated food allergy, only certain specific proteins are known to induce IgE sensitization. However, the immune system does not recognize the entire structure of the allergenic protein but instead responds to smaller sections called allergenic determinants or epitopes. Epitopes can be either continuous (a linear sequence of amino acids) or discontinuous (dependent upon the three-dimensional conformational structure of the protein) (16). Because food allergens are often stable in heat processing and digestion, it has been hypothesized that linear epitopes are more important with food allergens than might be the case with environmental allergens that are primarily inhaled (17). However, recent evidence with *Ara h* 1, the major peanut allergen, suggests that this hypothesis may not be true and that discontinuous epitopes are important in IgE binding (18). Although a thorough discussion of the immunological mechanisms of IgE-mediated food allergies is beyond the scope of this review, a two-phase process of sensitization and elicitation is involved (19). In the sensitization process, proteins are processed by proteolysis in an antigen-presenting cell. The peptides that result then react with T cells to provoke B cells to switch to production of allergen-specific IgE antibodies.

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

Once the allergen-specific IgE antibodies are attached to mast cells and basophils, the interaction of IgE-binding epitopes on these same proteins with the cell-bound IgE antibodies elicits the release of histamine and the other mediators of the allergic reactions. The allergen-specific IgE antibodies can bind to either linear or conformational epitopes depending upon the particular allergen involved. However, T cell epitopes are most likely exclusively linear and sensitization to a novel protein could not occur without the involvement of T cell epitopes. Although, if a novel protein introduced through agricultural biotechnology is identical to an existing allergen or is crossreactive with an existing allergen, then both the linear and conformational epitopes recognized by the cell-bound IgE could be important.

The use of six contiguous, identical amino acids as a match was predicated upon observations that the minimal IgE-binding epitopes of *Ara h* 1 and *Ara h* 2 involve six contiguous amino acids (20, 21). The minimum peptide length for a T cell–binding epitope is probably eight contiguous amino acids (9). Since this approach assesses the entire protein sequence, it is not based upon the identity of amino acid sequences just to known T cell– and B cell–binding epitopes of known allergens. Thus, this approach may (and probably will) identify matching sequences that are unrelated to the allergenic potential of the novel proteins. However, specific serum screening as described below should be able to eliminate clinically insignificant matches.

The criterion of 35% overall structural homology is intended to identify proteins that share similar functions. Many common plant allergens fall within a few functional categories (22). The pathogenesis-related proteins of several different types are prominently involved (22). If the novel proteins introduced into foods developed through agricultural biotechnology fall into functional categories that contain known food allergens, they are likely to have greater than 35% overall structural homology with these known allergens. In such situations, great caution must be exercised in assessing the potential allergenicity of these particular proteins.

If the sequence homology tests are positive, the conclusion might be reached that the novel protein is likely to be allergenic. In such situations, the commercial development of that particular modified crop might be halted as a result. Of course, the results of the sequence homology tests could be confirmed with specific serum screening, which would provide an even more reliable result. This may be advisable in situations where one of the sequence homology tests appears to yield a false positive result, such as an irrelevant match of six contiguous amino acids.

Specific Serum Screening

If the gene is derived from a known allergenic source or if the search for sequence homology identifies a match with a known allergenic source, then an assessment of the immunoreactivity of the novel protein with IgE antibodies from the sera of individuals allergic to the source material can be conducted, if desired, as noted above (11). However, since the structures of all of the allergens from all allergenic sources are not yet known, specific serum screening is required in every case where

the gene is derived from a known allergenic source (11). The typical approach is to bind the novel protein to a solid phase and then use blood serum from individuals known to be allergic to the specific allergenic source to determine if the allergen-specific IgE in the serum reacts with epitopes on the novel protein. The availability of sera from well-characterized patients is an important and sometimes challenging issue. Depending on the allergenic source, well-characterized human blood serum may be difficult to obtain. Sera would be considered well characterized if the patient had a positive and convincing history of allergy to the gene source, had a positive skin prick test or radio-allergosorbent test to an extract of the gene source, and ideally had a positive clinical challenge trial with the source material.

Another concern with specific serum screening is the possibility of clinically insignificant immunoreactivity. Many plant proteins contain carbohydrate moieties. The existence of IgE binding to carbohydrates is a well-known phenomenon (23). Some investigators believe these crossreactive carbohydrate determinants are clinically insignificant (23). Thus, the possibility of IgE binding to carbohydrate determinants must be excluded during specific serum screening (11).

A positive specific screening test would certainly raise concerns about the possible allergenicity of the novel protein. Of course, such tests could be discounted by additional in vivo testing in allergic patients, as was initially recommended in the IFBC/ILSI decision tree (9). However, obtaining ethics board approval for in vivo testing (skin prick tests and/or double-blind placebo-controlled food challenges) with genetically modified foods has proven difficult in some countries. Therefore, in vivo testing was not included as part of the FAO/WHO decision tree (11). In most circumstances, a positive result with specific serum screening will suggest that the novel protein is likely allergenic, and further commercial development will likely cease.

Targeted Serum Screening

In the case of negative or equivocal results in the specific serum screening as described above, the novel food should be investigated further using targeted serum screening (11). Targeted serum screening would also be used when the gene source has no history of allergenicity and has no sequence homology to known allergens (11).

In targeted serum screening, human blood serum is obtained from individuals who are allergic to materials that are broadly related to the source of the gene. Even if the gene source itself is not known to be allergenic, the possibility exists that the source material may contain proteins that are crossreactive with allergens from related sources. If the structure of such allergens is known, the crossreactivity will likely be evident in the sequence homology testing. However, since the structures of all of the allergens from all sources are not yet known, targeted serum screening is advisable. The FAO/WHO approach suggests several broad categories for targeted serum screening: monocots, dicots, invertebrates, vertebrates, and molds. As an example, if the source of a gene was a monocot with no history of

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

allergenicity, targeted serum screening would dictate that the immunoreactivity of the novel protein be assessed with sera from individuals with known allergies to other monocots such as grass pollen. The same caveats regarding the importance of well-characterized sera and exclusion of clinically insignificant IgE binding also apply to targeted serum screening. If the gene source is bacterial, then targeted serum screening is not conducted because bacterial proteins are rarely allergenic due to the low exposure levels and lack of allergic sensitization to these proteins.

If targeted serum screening is positive, then the novel protein is considered as likely allergenic. If targeted serum screening is negative, then further testing, including resistance to pepsin and immunoreactivity in animal models, is conducted.

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

Resistance to Pepsin

Proteolytic stability is a useful criterion in the assessment of the protein's allergenic potential. To become allergenic, a protein must reach the intestinal tract in a form that is sufficiently intact to provoke the immune system. If the protein is rapidly digested, that prospect seems unlikely. In simulated gastric and intestinal digestive models, known food allergens exhibited greater proteolytic stability than known nonallergenic food proteins (24). Many of the novel proteins introduced into foods produced through agricultural biotechnology were also rapidly digested in these same model systems (24). For example, the enzyme transferred into soybeans to make them tolerant to the herbicide glyphosate is rapidly digested in vitro (25) and is therefore unlikely to induce allergic sensitization.

The FAO/WHO decision-tree approach advocates use of resistance to proteolysis with pepsin as a comparative measure of digestive stability for novel proteins introduced into foods through agricultural biotechnology (11). Obviously, human digestion is individually variable, and this pepsin resistance assay should not be construed to be predictive of the digestive stability of a novel protein in all humans. However, the comparative resistance to pepsin proteolysis is likely a reasonable comparative measure in the allergenicity assessment. Novel proteins that are resistant to pepsin are more likely to become allergenic than proteins that are rapidly hydrolyzed by pepsin. Pepsin resistance is probably not a perfect indicator of allergenic potential. Some allergens in fresh fruits and vegetables are known to be sensitive to proteolysis (26). These particular allergens tend to be crossreactive with known pollen allergens (26) and would thus likely be discovered in the sequence homology testing. The FAO/WHO decision tree provides a specific protocol for the pepsin resistance test (11). Use of a standardized protocol is important so that comparative data can be obtained.

Animal Models

Well-validated animal models do not exist for prediction of the allergenicity of novel proteins. Several animal models, especially the Brown Norway rat and

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

several different mouse strains, appear to be promising (27–30). Despite the lack of a well-validated animal model, the FAO/WHO decision-tree approach advocated the use of animal models. This was probably done in an effort to stimulate more research on the development of animal models suitable for this purpose. Certainly, further research is needed on the development of animal models to assess and improve their predictive accuracy.

The ideal animal model should incorporate several attributes:

1. sensitization and challenge should be via the oral route since the natural digestive and gastrointestinal barriers can only be considered with this approach;
2. preferably, no use of adjuvants since the focus should be on the intrinsic allergenicity of the protein itself;
3. the test animal should produce a significant amount of IgE or other Th2-specific antibody class;
4. the test animal should tolerate most food proteins, especially proteins that are known nonallergens, such as Rubisco;
5. the test animal should develop allergen-specific IgE antibodies on oral exposure to known food allergens;
6. the test should be relatively easy and reproducible.

Unfortunately, oral exposure to food proteins in rodents typically results in immunological tolerance (31, 32). However, recent research has led to the development of several approaches that may prove useful in the development of a validated animal model. Repeated enteral administration of proteins in combination with adjuvants indicates that allergic sensitization can be achieved in mice with certain protocols (29, 33). Using specific sensitization protocols, allergic sensitization in mice with oral administration has been achieved to cows' milk (29) and peanut proteins (33) using cholera toxin as an adjuvant. Oral feeding of mice with casein or ovalbumin as a constituent of the diet without adjuvant administration only resulted in allergic sensitization to casein (34). Using intraperitoneal administration of proteins, Dearman et al. (35) showed that the characteristic antibody (IgG and IgE) isotype profiles are different for ovalbumin (an allergenic protein) and bovine serum albumin (a weakly allergenic protein). The Brown Norway rat is known to produce high levels of IgE when provoked with antigen. Knippels et al. (27) were able to elicit IgE responses to known food allergens in cows' milk and eggs by daily oral gavage dosing of Brown Norway rats. However, further validation of the Brown Norway rat model using food proteins that are not allergens would be helpful. The Beagle dog may also be a useful animal model to consider in further research (30).

In the FAO/WHO decision tree, novel proteins that are resistant to pepsin and able to stimulate IgE responses in suitable animal models are more likely to become allergens than proteins that are rapidly hydrolyzed by pepsin and fail to stimulate immune responses in these same animal models.

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

Level of Expression of the Novel Protein

In many cases, the novel proteins are expressed at very low levels in foods produced through agricultural biotechnology. Certainly in the cases of insect resistance and herbicide tolerance, the current products on the market contain rather low levels of the novel proteins, but those levels are sufficient to provide the modified crop with the enhanced agronomic benefits.

Metcalfe et al. (9) suggested that the level of expression was probably an important factor to consider in assessing the allergenicity of foods produced through agricultural biotechnology. However, the level of expression of the novel protein has not actually been included in various decision-tree strategies, including the recent FAO/WHO decision tree.

Emerging evidence suggests that a threshold dose does exist below which allergic individuals will not react adversely to the offending food. Experience with double-blind, placebo-controlled trials suggests that the threshold dose for peanut protein is in the neighborhood of 1 to 20 mg (36–38). Apparently, if an allergenic protein was expressed in a food produced through agricultural biotechnology at levels well below 1 mg per serving, the hazard for allergic consumers would be minimal.

Since the genes transferred through agricultural biotechnology are often obtained from sources with no history of allergenicity, the threshold dose for sensitization to a novel protein is also an important consideration. Unfortunately, very little information exists on the threshold dose for sensitization. As this aspect of the potential allergenicity of genetically modified foods is extremely important, the lack of information on the threshold dose for sensitization has prevented the inclusion of this criterion in various decision-tree approaches (11). The best information may come from studies on exclusively breast-fed infants; but as reviewed elsewhere, these data are very difficult to interpret because dietary exposures cannot be precisely controlled in infants (3).

APPLICATION OF ALLERGENICITY ASSESSMENT

As noted earlier, allergenicity assessments have been conducted on all commercial genetically modified foods that are currently on the market. One particular example demonstrates the value of this approach. In the early 1990s, Pioneer Hi-Bred International, now a division of DuPont, developed a high-methionine variety of soybeans by cloning the gene for a high-methionine protein from Brazil nuts into soybeans. Soybeans are inherently deficient in methionine. Thus, farmers must supplement animal diets with methionine when feeding soybean meal to nonruminant farm animals. The high-methionine protein from Brazil nuts was expressed at a reasonably high level in the novel variety of soybeans. At the time of this development, the allergenicity of Brazil nuts was well established (39), but the identity of the allergens in Brazil nuts was not known. Nordlee et al. (40) evaluated the possible allergenicity of the novel soybeans and the purified

high-methionine protein using blood sera from individuals with documented Brazil nut allergy. Specific serum screening showed that the gene obtained from the Brazil nut likely encoded for a major Brazil nut allergen (*Ber e* 1). This possibility was confirmed by positive skin prick tests on three of the Brazil nut–allergic individuals (40). As a result, Pioneer Hi-Bred International decided not to commercialize this variety of soybeans.

CONCLUSION

Foods produced through agricultural biotechnology should be assessed for their potential allergenicity. Strategies do exist for allergenicity assessments. However, further research will aid in the implementation of these strategies particularly with respect to an improved database on the amino acid sequences and epitopes of known food allergens and the development of well-validated animal models. With the application of these strategies to foods produced through agricultural biotechnology, consumers and regulators should be confident that the likelihood of allergenicity of the novel foods that pass the screens is quite low.

Visit the Annual Reviews home page at www.AnnualReviews.org

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

LITERATURE CITED

1. IFT Expert Report on Biotechnology and Foods. 2000. Benefits and concerns associated with recombinant DNA biotechnology-derived foods. *Food Technol.* 54:61–80
2. IFT Expert Report on Biotechnology and Foods. 2000. Introduction. *Food Technol.* 54:124–36
3. Taylor SL, Hefle SL. 2001. Will genetically modified foods be allergenic? *J. Allergy Clin. Immunol.* 107:765–71
4. Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, et al. 2000. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287:303–5
5. Food and Agriculture Organization. 1996. *Biotechnology and Food Safety: Report of a Joint FAO/WHO Consultation*. Rome: Food and Agric. Organ. of United Nations
6. World Health Organization. 2000. *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation*. Geneva: World Health Organ.
7. Organization for Economic Cooperation and Development. 1992. *Safety Evaluation of Foods Derived by Modern Biotechnology—Concepts and Principles*. Paris: Organ. Econ. Coop. Dev.
8. Beever DE, Kemp CF. 2000. Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutr. Abstr. Rev. Series B—Livestock Feeds and Feeding* 70:175–82
9. Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, et al. 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Crit. Rev. Food Sci. Nutr.* 36:S165–86
10. Wal JM. 1998. Strategies for assessment and identification of allergenicity in (novel) foods. *Intl. Dairy J.* 8:413–23
11. Food and Agriculture Organization. 2001.

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

Evaluation of the Allergenicity of Genetically Modified Foods: Report of a Joint FAO/WHO Expert Consultation. Rome: Food and Agric. Organ. of United Nations

12. Calkoven PG, Aalbers M, Koshte VL, Pos O, Oei HD, et al. 1987. Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 42:382–90
13. Food and Agriculture Organization. 1995. *Report of the FAO Technical Consultation on Food Allergies*. Rome: Food and Agric. Organ. of United Nations
14. Hefle SL, Nordlee JA, Taylor SL. 1996. Allergenic foods. *Crit. Rev. Food Sci. Nutr.* 36:S69–89
15. Gendel SM. 1998. The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods. *Adv. Food Nutr. Res.* 42:45–62
16. Taylor SL, Lehrer SB. 1996. Principles and characteristics of food allergens. *Crit. Rev. Food Sci. Nutr.* 36:S91–118
17. Taylor SL, Lemanske RF, Bush RK, Busse WW. 1987. Chemistry of food allergens. In *Food Allergy*, ed. RK Chandra, pp. 21–44. St. John's, Newfoundland: Nutr. Res. Educ. Found.
18. Shin DS, Compadre CM, Maleki SJ, Kopper RA, Sampson H, et al. 1998. Biochemical and structural analysis of the IgE binding sites on Ara h1, an abundant and highly allergenic peanut protein. *J. Biol. Chem.* 273:13753–59
19. Mekori YA. 1996. Introduction to allergic disease. *Crit. Rev. Food Sci. Nutr.* 36:S1–18
20. Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, et al. 1997. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *Eur. J. Biochem.* 245:334–39
21. Stanley JS, King N, Burks AW, Huang SK, Sampson H, et al. 1997. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch. Biochem. Biophys.* 342:244–53
22. Breiteneder H, Ebner C. 2000. Molecular and biochemical classification of plant-derived food allergens. *J. Allergy Clin. Immunol.* 106:27–36
23. Aalberse RC, Van Ree R. 1997. Crossreactive carbohydrate determinants. *Clin. Rev. Allergy Immunol.* 15:375–87
24. Astwood JD, Leach JN, Fuchs RL. 1996. Stability of food allergens to digestion in vitro. *Nat. Biotechnol.* 14:1269–73
25. Harrison LA, Bailey MR, Naylor MW, Ream JE, Hammond BG, et al. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. *J. Nutr.* 126:728–40
26. Moneret-Vautrin DA, Kanny G, Rance F, Lemerdy P. 1997. Les allergens vegetaux alimentaires. Allergies associees et reactions croisees. *Rev. Fr. Allergol. Immunol.* 37:316–24
27. Knippels LMJ, Penninks AH, Spanhaak S, Houben GF. 1998. Oral sensitization to food proteins: a Brown Norway rat model. *Clin. Exp. Allergy* 28:368–75
28. Knippels LMJ, Felius AA, van der Kleij HPM, Penninks AH, Koppelman SJ, et al. 2000. Comparison of antibody responses to hen's egg and cow's milk proteins in orally sensitized rats and food-allergic patients. *Allergy* 55:251–58
29. Li X, Schofield BH, Huang CK, Kleiner GI, Sampson HA. 1999. A murine model for IgE-mediated cow's milk hypersensitivity. *J. Allergy Clin. Immunol.* 103:206–14
30. Ermel RW, Kock M, Griffey SM, Reinhart GA, Frick OL. 1997. The atopic dog: a model for food allergy. *Lab. Animal Sci.* 47:40–49
31. Steinmann J, Wottge HU, Muller-Ruchholtz W. 1990. Immunogenicity testing of food proteins: in vitro and in vivo trials in rats. *Intl. J. Allergy Appl. Immunol.* 91:62–65

32. Strobel S, Ferguson A. 1984. Immune responses to fed protein antigens in mice. Systemic tolerance or priming is related to age at which antigen is first encountered. *Pediatr. Res.* 18:588–94
33. Li X, Serebrisky D, Lee SJ, Huang CK, Bardina L, et al. 2000. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J. Allergy Clin. Immunol.* 106:150–58
34. Ito K, Ohara KI, Murosaki S, Nishimura H, Shimokata T, et al. 1997. Murine model of IgE production with a predominant Th2-response by feeding protein antigen without adjuvants. *Eur. J. Immunol.* 27:3427–37
35. Dearman RJ, Caddick H, Basketter DA, Kimber I. 2000. Divergent antibody isotype responses induced in mice by systemic exposure to proteins: a comparison of ovalbumin with bovine serum albumin. *Food Chem. Toxicol.* 38:351–60
36. Hourihane JO'B, Kilburn SA, Nordlee JA, Hefle SL, Taylor SL, et al. 1997. An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study. *J. Allergy Clin. Immunol.* 100:596–600
37. Nelson HS, Lahr J, Rule R, Bock A, Leung D. 1997. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J. Allergy Clin. Immunol.* 99:744–51
38. Rance F, Dutau G. 1999. Peanut hypersensitivity in children. *Pediatr. Pulmonol.* (Suppl.) 18:165–67
39. Arshad SH, Malmberg E, Krapf K, Hide DW. 1991. Clinical and immunological characteristics of Brazil nut allergy. *Clin. Exp. Allergy* 21:373–76
40. Nordlee JA, Taylor SL, Townsend JA, Thomas LA, Bush RK. 1996. Identification of a Brazil-nut allergen in transgenic soybeans. *N. Engl. J. Med.* 334:688–92

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

Annual Review of Pharmacology and Toxicology
Volume 42, 2002

CONTENTS

REGULATION OF CYP3A GENE TRANSCRIPTION BY THE PREGNANE X RECEPTOR, *Bryan Goodwin, Matthew R. Redinbo, and Steven A. Kliewer* 1

INHIBITION OF CARCINOGENESIS BY TEA, *Chung S. Yang, Pius Maliakal, and Xiaofeng Meng* 25

COX-2: A TARGET FOR COLON CANCER PREVENTION, *Lawrence J. Marnett and Raymond N. DuBois* 55

CYTOKINE MODULATORS AS NOVEL THERAPIES FOR ASTHMA, *Peter J. Barnes* 81

PROTEIN ALLERGENICITY ASSESSMENT OF FOODS PRODUCED THROUGH AGRICULTURAL BIOTECHNOLOGY, *Steve L. Taylor* 99

CHALLENGES FOR BIOMEDICAL INFORMATICS AND PHARMACOGENOMICS, *Russ B. Altman and Teri E. Klein* 113

MOLECULAR PSYCHOLOGY: ROLES FOR THE ERK MAP KINASE CASCADE IN MEMORY, *J. Paige Adams and J. David Sweatt* 135

GLUTAMATERGIC MECHANISMS IN SCHIZOPHRENIA, *Guochuan Tsai and Joseph T. Coyle* 165

MOLECULAR BASIS OF ENVIRONMENTALLY INDUCED BIRTH DEFECTS, *Richard H. Finnell, Janée Gelineau-van Waes, James D. Eudy, and Thomas H. Rosenquist* 181

PROTEIN TYROSINE PHOSPHATASES: STRUCTURE AND FUNCTION, SUBSTRATE SPECIFICITY, AND INHIBITOR DEVELOPMENT, *Zhong-Yin Zhang* 209

AKAP-MEDIATED SIGNAL TRANSDUCTION, *Jennifer J. Carlisle Michel and John D. Scott* 235

CELLULAR MECHANISMS FOR THE REPRESSION OF APOPTOSIS, *Carl D. Bortner and John A. Cidlowski* 259

SIGNAL TRANSDUCTION BY CELL ADHESION RECEPTORS AND THE CYTOSKELETON: FUNCTIONS OF INTEGRINS, CADHERINS, SELECTINS, AND IMMUNOGLOBULIN-SUPERFAMILY MEMBERS, *R. L. Juliano* 283

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org
by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.

DESIGNING INHIBITORS OF CYCLIN-DEPENDENT KINASES, *Ian R. Hardcastle, Bernard T. Golding, and Roger J. Griffin* 325

DRUG EFFICACY AT G PROTEIN–COUPLED RECEPTORS, *Terry Kenakin* 349

GLYCOPEPTIDE ANTIBIOTIC RESISTANCE, *Jeff Pootoolal, John Neu, and Gerard D. Wright* 381

DIMERIZATION: AN EMERGING CONCEPT FOR G PROTEIN–COUPLED RECEPTOR ONTOGENY AND FUNCTION, *Stephane Angers, Ali Salahpour, and Michel Bouvier* 409

THE BINDING SITE OF AMINERGIC G PROTEIN–COUPLED RECEPTORS: THE TRANSMEMBRANE SEGMENTS AND SECOND EXTRACELLULAR LOOP, *Lei Shi and Jonathan A. Javitch* 437

STRUCTURE, FUNCTION, AND INHIBITION OF CHEMOKINES, *Elias J. Fernandez and Elias Lolis* 469

ALTERED DNA METHYLATION: A SECONDARY MECHANISM INVOLVED IN CARCINOGENESIS, *Jay I. Goodman and Rebecca E. Watson* 501

THE CHANGING FACE OF THE Na^+/H^+ EXCHANGER, NHE1: STRUCTURE, REGULATION, AND CELLULAR ACTIONS, *L. K. Putney, S. P. Denker, and D. L. Barber* 527

THE SPINAL PHOSPHOLIPASE-CYCLOOXYGENASE-PROSTANOID CASCADE IN NOCICEPTIVE PROCESSING, *Camilla I. Svensson and Tony L. Yaksh* 553

THE BIOCHEMISTRY AND PHYSIOLOGY OF S-NITROSOTHIOLS, *Neil Hogg* 585

INDEXES

Subject Index 601

Cumulative Index of Contributing Authors, Volumes 38–42 627

Cumulative Index of Chapter Titles, Volumes 38–42 630

ERRATA

An online log of corrections to *Annual Review of Pharmacology and Toxicology* chapters may be found at http://pharmtox.annualreviews.org/errata.shtml

Annu. Rev. Pharmacol. Toxicol. 2002.42:99-112. Downloaded from arjournals.annualreviews.org by Universita' degli Studi di Roma, Tor Vergata on 02/17/06. For personal use only.