

- eds), pp. 465–481, Butler and Tanner, Frome, UK
- 22 Hutchings, J.B. and Lillford, P.J. (1988) 'The Perception of Food Texture – The Philosophy of the Breakdown Path' in *J. Texture Stud.* 19, 103–115
 - 23 Boyar, M.M. and Kilcast, D. (1986) 'Electromyography as a Novel Method for Examining Food Texture' in *J. Food Sci.* 51, 859–860
 - 24 Jack, F.R., Piggott, J.R. and Paterson, A. (1993) 'Relationships Between Electromyography, Sensory and Instrumental Measures of Cheddar Cheese Texture' in *J. Food Sci.* 58, 1313–1317
 - 25 Mioche, L., Peyron, M.A. and Auroy, P. (1993) 'The Use of Intraoral Load Cells in the Study of Texture Perception' in *J. Texture Stud.* 24, 361–373
 - 26 Szczesniak, A.S. (1963) 'Classification of Textural Characteristics' in *J. Food Sci.* 28, 385–389
 - 27 Brandt, M.A., Skinner, E.Z. and Coleman, J.A. (1963) 'Texture Profile Method' in *J. Food Sci.* 28, 404–410
 - 28 Langstaff, S.A., Guinard, J.-X. and Lewis, M.J. (1991) 'Sensory Evaluation of the Mouthfeel of Beer' in *J. Am. Soc. Brew. Chem.* 49, 54–59
 - 29 Marshall, R.J. (1993) 'Physicochemical Properties Accounting for Cheese Texture' in *Acta Psychol.* 84, 69–77
 - 30 Peyron, M.A., Mioche, L. and Culioli, J. (1994) 'Bite Force and Sample Deformation During Hardness Assessment of Viscoelastic Models of Foods' in *J. Texture Stud.* 25, 59–76
 - 31 Vickers, Z.M. (1984) 'Crispness and Crunchiness – A Difference in Pitch?' *J. Texture Stud.* 15, 157–160
 - 32 Seymour, S.K. and Hamann, D.D. (1988) 'Crispness and Crunchiness of Selected Low Moisture Foods' in *J. Texture Stud.* 19, 79–95
 - 33 Rohde, F., Normand, M.D. and Peleg, M. (1993) 'Characterization of the Power Spectrum of Force–Deformation Relationships of Crunchy Foods' in *J. Texture Stud.* 24, 45–62
 - 34 Peleg, M. (1993) 'Do Irregular Stress–Strain Relationships of Crunchy Foods Have Irregular Periodicities?' in *J. Texture Stud.* 24, 213–227
 - 35 Mela, D.J. (1988) 'Sensory Assessment of Fat Content in Fluid Dairy Products' in *Appetite* 10, 37–44
 - 36 Kokini, J.L. (1985) 'Fluid and Semi-solid Food Texture and Texture–Taste Interactions' in *Food Technol.* 39, 86–92
 - 37 Richardson, N.J., Booth, D.A. and Stanley, N.L. (1993) 'Effect of Homogenization and Fat Content on Oral Perception of Low and High Viscosity Model Creams' in *J. Sens. Stud.* 8, 133–143
 - 38 Kokini, J.L. and Cussler, E.L. (1983) 'Predicting the Texture of Liquid and Melting Semi-solid Food' in *J. Food Sci.* 48, 1221–1225
 - 39 Daget, N., Joerg, M. and Bourne, M. (1987) 'Creamy Perception I: In Model Dessert Creams' in *J. Texture Stud.* 18, 367–388
 - 40 Stanley, N.L. and Taylor, L.J. (1993) 'Rheological Basis of Oral Characteristics of Fluid and Semi-solid Foods: A Review' in *Acta Psychol.* 84, 79–92
 - 41 Shama, F. and Sherman, P. (1973) 'Identification of Stimuli Controlling the Sensory Evaluation of Viscosity. II. Oral Methods' in *J. Texture Stud.* 4, 111–118
 - 42 Christensen, C.M. and Casper, L.M. (1987) 'Oral and Nonoral Perception of Solution Viscosity', in *J. Food Sci.* 52, 445–447
 - 43 Szczesniak, A.S. and Ilker, R. (1988) 'The Meaning of Textural Characteristics – Juiciness in Plant Foodstuffs' in *J. Texture Stud.* 19, 61–78
 - 44 Green, B.G. (1993) 'Oral Astringency: A Tactile Component of Flavor' in *Acta Psychol.* 84, 119–125
 - 45 Guinard, J.-X., Pangborn, R.M. and Lewis, M.J. (1986) 'The Time Course of Astringency in Wine Upon Repeated Ingestion' in *Am. J. Enol. Vitic.* 37, 184–189
 - 46 Johnson, K.O. and Hsiao, S.S. (1992) 'Neural Mechanisms of Tactile Form and Texture Perception' in *Annu. Rev. Neurosci.* 15, 227–250
 - 47 Breslin, P.A.S., Gilmore, M.M., Beauchamp, G.K. and Green, B.G. (1993) 'Psychological Evidence That Oral Astringency is a Tactile Sensation' in *Chem. Senses* 18, 405–417
 - 48 Green, B.G. and Lawless, H.T. (1991) 'The Psychophysics of Somatosensory Chemoreception in the Nose and the Mouth' in *Smell and Taste in Health and Disease* (Getchel, T.V., Doty, R.L., Bartoshuk, L.M. and Snow, J.B., Jr, eds), pp. 235–253, Raven Press
 - 49 Green, B.G. (1992) 'The Effect of Temperature and Concentration on the Perceived Intensity and Quality of Carbonation' in *Chem. Senses* 17, 435–450
 - 50 Szczesniak, A.S. and Khan, E.L. (1971) 'Consumer Awareness and Attitudes to Food Texture. I. Adults' in *J. Texture Stud.* 2, 280–295
 - 51 Birch, L.L. (1992) 'Children's Preferences for High-fat Foods' in *Nutr. Rev.* 50, 249–255
 - 52 Guinard, J.-X., Zoumas-Morse, C., Mori, L., Panyam, D. and Kilara, A. 'Effect of Sugar and Fat on the Acceptability of Vanilla Ice Cream' in *J. Dairy Sci.* (in press)
 - 53 Lillford, P.J. (1991) 'Texture and Acceptability of Human Foods' in *Feeding and the Texture of Food (Society for Experimental Biology Seminar Series 44)* (Vincent, J.F.V. and Lillford, P.J., eds), pp. 231–243, Cambridge University Press

Review

Food allergy represents a largely avoidable threat. Technologies are presently available or are under development that better define the molecules (allergens) responsible for food allergy, better detect the presence of allergens, and can reduce or even eliminate specific allergens from the food supply. The incidence of food allergy and fatal anaphylaxis triggered by food allergens is expected to decline as a result of the application of a combination of key technologies such as biotechnology, bioinformatics and immunodiagnostics, and of the Internet, as well as following the development of well-defined science-based public health policies.

A review of recent technological advances that can help prevent food allergy is presented in this article. The use of recombinant DNA techniques to identify and characterize

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Preventing food allergy: Emerging technologies

James D. Astwood and Roy L. Fuchs

food allergens has been important in these advances. Recombinant DNA techniques have led to improved methods of allergen detection, more accurate allergy diagnoses and new strategies for rational and safe immunotherapy (see Glossary). Recombinant allergens are also accelerating the development of diagnostic capabilities that are needed for the detection of accidental contamination of processed foods by unwanted allergens. Recombinant DNA techniques have also been used to effect the ultimate food allergy prevention scheme – the elimination of allergens from the food supply. At the same time, concerns about the use of recombinant DNA techniques in the development

Glossary

Anaphylaxis: Generalized inflammatory immune reaction resulting in vasodilation and contraction of the smooth muscle cells, including those of the bronchus. An IgE-mediated response to a foreign protein in a sensitized individual that may be severe enough to be life threatening.

Atopy: Foreign, unusual or out of place; used synonymously with allergy and hypersensitivity. A form of immunological reactivity in which reaginic antibody (IgE) is readily produced in response to exposure to allergens. Occasionally used to describe immediate hypersensitivities with strong familial tendencies.

Cytokine: Hormone-like protein, secreted by cells such as lymphocytes and macrophages, that acts as a molecular signal for communication between cells of the immune system (also 'lymphokine').

IgE: A specific class or isotype of immunoglobulin that is produced in response to allergens and that binds to specific receptors on mast cells. The primary antibody in type I (immediate) hypersensitivity.

Immunotherapy: Any type of treatment that targets the immune system. In allergy, it is a treatment that progressively increases a dose of allergen in order to induce an immune response that is characterized by tolerance or desensitization to the allergen.

Epitope: A single antigenic determinant. Functionally, it is the portion of an antigen that combines with an antibody such as IgE. The T-cell epitope is a segment of an allergen that binds to a T cell, and subsequently induces an allergic response.

of new food crop varieties have been articulated, and approaches to address these concerns have been described and implemented.

General aspects of food allergy

Excellent reviews have described the clinical and experimental aspects of food allergy¹⁻³. Food allergy is a distinct class of adverse reaction to foods: it is defined as an immunological and IgE-mediated hypersensitivity⁴. Thus, food allergy should not be confused with food intolerances that may be metabolic (e.g. lactose intolerance), pharmacological (e.g. scombroid fish poisoning from histamines), toxic (e.g. shellfish poisoning from dinoflagellate saxitoxin) or psychological⁵. Although a handful of foods or food groups accounts for the vast majority of food allergy cases, it has been calculated recently that over 100 different foods or food components have produced documented adverse reactions⁶. The structural and immunological properties of major food allergens were reviewed recently in *Trends in Food Science & Technology* by Matsuda and Nakamura⁷. Peanuts, soybeans, tree nuts, milk, eggs, fish, crustaceans and wheat are responsible for 90% of food-allergic reactions in the USA⁶. For simplicity, coverage in this article is restricted to IgE-mediated allergies (also known as type I hypersensitivity) and their causative agents. However, it is anticipated that similar conclusions will emerge for other food-protein-related gastrointestinal disorders such as coeliac disease, a relatively rare condition that is caused by the consumption of foods containing wheat, rye or barley flour (e.g. bread).

Although there are clear indications that individual propensity towards allergy has a large genetic component, attempts to define a human 'atopy' gene have been

inconclusive⁸. For example, it has been suggested that elevated serum IgE levels and bronchial hypersensitivity reactions are associated with the large arm of human chromosome 5. High levels of circulating IgE might predispose individuals to IgE-mediated allergies. At the same time, the responses of mast cells to IgE may be associated with mutations in the gene coding for the β -subunit of the high-affinity IgE receptor, which is located on the large arm of chromosome 11. IgE receptors are found on mast cells; they play a pivotal role in allergy because allergen-IgE binding to mast cell IgE receptors triggers the cascade of inflammatory responses. This cascade, mediated by histamine and cytokines, results in mucosal inflammation and characteristic type I allergic reactions such as anaphylaxis. Interestingly, the gene for interleukin 4 (IL-4), a cytokine that is thought to be an important mediator, is also located on chromosome 11. It is tempting to speculate that a single region of chromosome 11 is a genetic 'hot spot' that confers a propensity towards allergy.

The diagnosis of food allergy relies on the use of case histories, clinical examinations, skin tests, *in vitro* assays, and ultimately the double-blind placebo-controlled food challenge (DBPCFC), as recently reviewed by the European Academy of Allergology and Clinical Immunology subcommittee on adverse reactions to food⁹. The exact prevalence of food allergy is not well understood, as few exhaustive epidemiological studies have been completed. Estimates suggest that up to 5% of children and 2% of adults are afflicted by bona fide food allergies².

Once an individual has a confirmed case of food allergy, the most common course of 'treatment' is avoidance (elimination diet) of the allergenic food(s)^{3,9}. Most food-allergic individuals react to a limited number of food types (one to three). Nevertheless, it is well recognized that there are cross-reactivities between related and unrelated food groups. Also, there is an association of certain food allergies with certain pollen allergies (for example, many vegetable food allergies are associated with allergy to birch pollen). However, these associations are not yet well understood at either the molecular or the clinical level, although it has been hypothesized that pollen serves as the primary sensitizing agent for such food allergies¹⁰. Therefore, avoidance of only the food(s) causing the allergy remains the most commonly recommended strategy for food allergy management. At the same time, there has been speculation that exposure of young children to certain types of foods can enhance an individual's propensity to food allergy later in life, although no evidence to support this hypothesis has yet emerged¹¹. At present, recommendations vary with respect to when these foods should be introduced into the diets of infants. Thus, prevention of food allergy boils down to avoidance of those foods that cause allergy, either by naive infants or by food-allergic individuals.

Recombinant DNA technology, bioinformatics and allergen characterization

Over 200 proteinaceous allergens (from all sources, food and non-food) have been identified and characterized

using molecular biology techniques¹²⁻¹⁴. This means that many allergen genes, most often in the form of a cDNA, have been isolated and sequenced; thus, the resulting amino acid sequence of many allergens is known. This molecular biology revolution has led to the development of publicly accessible databases of gene sequence information. The databases (i.e. GenBank, PIR, EMBL and SwissProt) are themselves useful tools because they contain entries of nucleotide sequences, amino acid sequences, bibliographic citations, and other information against which new DNA and protein sequences can be compared for similarity. Public databases can be accessed through the Internet at the National Center for Biotechnology Information World Wide Web site (<http://www.ncbi.nlm.nih.gov/>). As more and more bio-information accumulates, fundamental molecular facts such as common molecular determinants of allergenicity and evolutionary relationships among allergens should become apparent.

Recombinant allergens have a variety of other clinical and experimental applications¹⁵. Molecular information about allergens will lead to a better understanding of the pathogenesis of the atopic immune response. Recombinant techniques enable the production of relatively large quantities of highly purified allergens in heterologous protein expression systems (Fig. 1). Highly purified allergens may prove to be extremely valuable both as diagnostic and as therapeutic agents because they are free from protein contaminants, which are an unavoidable consequence of standard protein purification methods¹².

At present, immunotherapy for food allergy is uncommon¹⁶, although some success has been achieved for peanut allergy¹⁷. However, immunotherapy for allergy to pollens, molds, mites and venoms is more common¹⁸. In classical immunotherapy, crude protein extracts of allergens are administered to an individual at increasing doses, with the intention of inducing in the individual either tolerance to or protection against the original allergen. Protection is thought to occur through an increase in allergen-specific IgG, induction of anti-idiotypic antibodies, activation of suppressor T cells, suppression of IgE formation, and other unknown mechanisms¹⁸. Unfortunately, the use of crude extracts can cause some individuals to become sensitized to antigens and allergens that they could tolerate before immunotherapy. In addition, immunotherapy can induce allergic reactions, and anaphylaxis can occur. Improved, highly purified immunotherapy reagents should reduce the risk of such adverse reactions. In addition, the availability of recombinant allergens, especially those for which T-cell epitopes have been mapped, may mean that highly specific peptide-based immunotherapy can be attempted¹⁹. Although this approach remains controversial¹², T-cell epitope immunotherapy is attractive because there may be less potential for anaphylaxis and other allergic side effects.

Detection of food allergens: immunodiagnosics

Because it is possible for food-allergic individuals to avoid food allergens by adjusting their diet, it is necessary to assure that allergens do not appear in foods in which

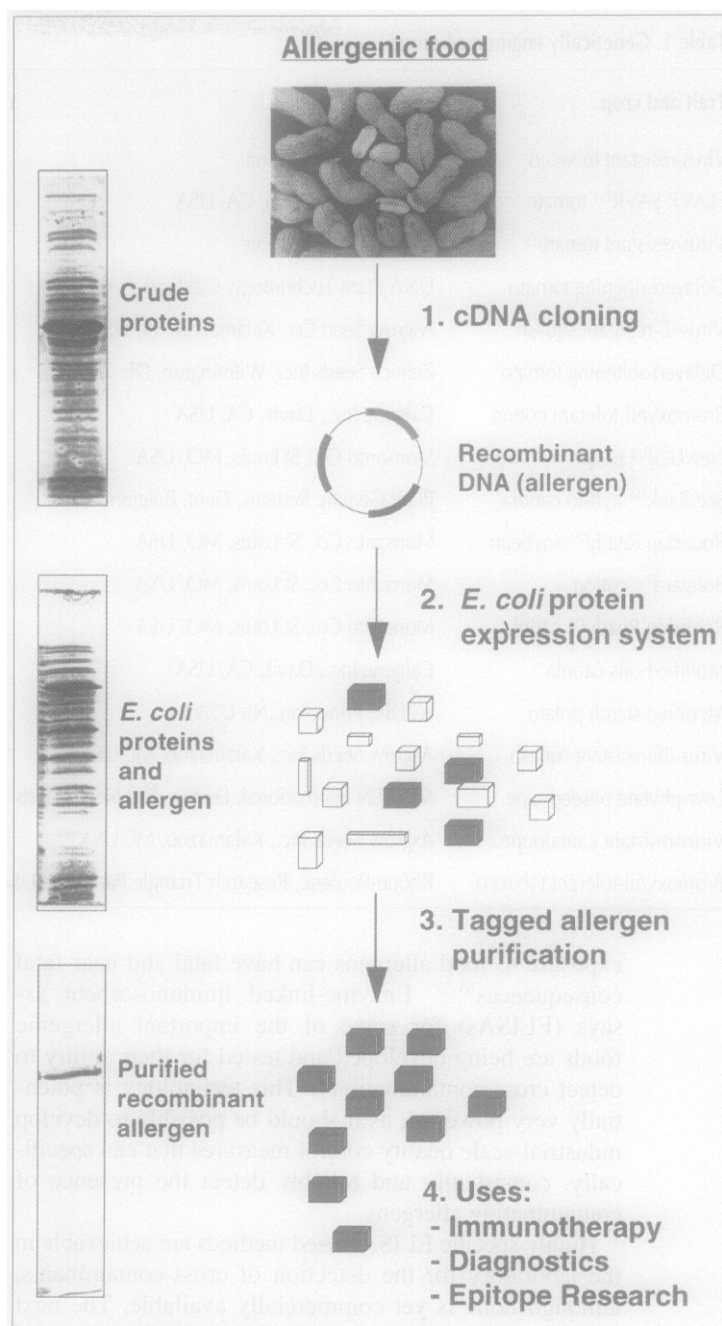


Fig. 1

Application of recombinant allergens. (1), The DNA sequence of the allergen gene is determined and complementary (copy or c) DNA is made. (2), The allergen cDNA is incorporated into an *Escherichia coli* plasmid vector designed for protein expression and containing a specific fusion 'tag' such as the maltose-binding protein (New England Biolabs Ltd, Cambridge, MA, USA). The allergen protein is expressed as a fusion to the maltose-binding protein. (3), This allows the rapid purification of recombinant 'tagged' allergens in a one-step maltose affinity chromatography procedure. Tags can be removed by cleavage with factor Xa, if desired. To the left of the stages of this process are photographs of sodium dodecyl – polyacrylamide gel electrophoresis gels of crude proteins, *E. coli* proteins containing an expressed tagged allergen, and subsequently purified recombinant tagged allergen.

they would not be expected to be present. This is important from the point of view of both ingredient labelling of processed foods and the avoidance of food cross-contamination. It is already well established that accidental

Table 1. Genetically engineered crops

Trait and crop	Organization	Year of introduction
Virus-resistant tobacco	Government of China	1993
FLAVR SAVR™ tomato	Calgene Inc., Davis, CA, USA	1994
Virus-resistant tomato	Government of China	1994
Delayed-ripening tomato	DNA Plant Technology, Oakland, CA, USA	1995
Virus (I)-resistant squash	Asgrow Seed Co., Kalamazoo, MI, USA	1995
Delayed-softening tomato	Zeneca Seeds Inc., Wilmington, DE, USA	1995
Bromoxynil-tolerant cotton	Calgene Inc., Davis, CA, USA	1995
NewLeaf™ potato	Monsanto Co., St Louis, MO, USA	1995
SeedLink™ hybrid canola	Plant Genetic Systems, Gent, Belgium	1996
Roundup Ready™ soybean	Monsanto Co., St Louis, MO, USA	1996
Bollgard™ cotton	Monsanto Co., St Louis, MO, USA	1996
Roundup Ready™ canola	Monsanto Co., St Louis, MO, USA	1996
Modified-oils canola	Calgene Inc., Davis, CA, USA	1996
Modified-starch potato	AVEBE, Princeton, NJ, USA	1996–1997
Virus (II)-resistant squash	Asgrow Seeds Inc., Kalamazoo, MI, USA	1996–1997
Low-phytate oilseed rape	MOGEN International, Leiden, The Netherlands	1996–1997
Virus-resistant cantaloupe	Asgrow Seeds Inc., Kalamazoo, MI, USA	1996–1997
Bromoxynil-tolerant tobacco	Rhône-Poulenc, Research Triangle Park, NC, USA	1996–1997

exposure to food allergens can have fatal and near-fatal consequences^{20–23}. Enzyme-linked immunosorbent assays (ELISAs) for some of the important allergenic foods are being developed and tested for their ability to detect cross-contamination²⁴. This technology is potentially very powerful, as it should be possible to develop industrial-scale quality control measures that can specifically, consistently and reliably detect the presence of contaminating allergens.

Highly specific ELISA-based methods are achievable in the laboratory for the detection of cross-contaminants, although none is yet commercially available. The next step in safety assurance will be the development of on-site immunodiagnostic kits. The advantage of on-site immunodiagnostics will be the fact that allergen detection will be timely, occurring before large quantities of contaminated products reach the marketplace. At the same time, immunodiagnostic kits should make allergen detection simple to perform in the commercial environment. Indeed, the use of immunodiagnostic methods for the detection of pesticide levels and microbial contamination in foods is already a 150-million-dollar business in the USA²⁵. For example, proprietary on-site immunodiagnostics have been developed for the detection of low-abundance specific proteins that are expressed by transgenic insect-protected crops.

Recombinant allergens may also have a role in the development of ELISAs that are highly allergen specific. Presently, ELISAs are available for detecting crude food proteins that contain both allergens and non-allergens. Allergen-specific ELISAs based on several important recombinant allergens may result in more specific and accurate immunodiagnostics for assessing food safety.

Food allergy and transgenic plants

The development of transgenic plants was first reported in 1984 (Ref. 26). Since then, essentially all economically important crops have been genetically engineered. Thus, opportunities to develop more sustainable production practices^{27,28}, safer and healthier foods²⁹, biopharmaceuticals³⁰, and bio-industrial commodities³¹ are now being realized. Most of the traits introduced into crops result from the expression of one or more proteins that are not normally expressed in the non-transgenic crop plants. Several transgenic plant products have been commercialized and many others will arrive in the marketplace in the near future (Table 1). Before market introduction, each of these products is subjected to extensive food, feed and environmental safety assessments, including an assessment of the allergenic potential of the proteins encoded by the introduced genes.

The prevention of unwanted allergens from entering the food supply via transgenic plants depends on several

common-sense principles. The transfer of known allergens into plants must be avoided. This statement is true whether recombinant DNA techniques or traditional plant breeding methods are used to develop a new plant variety. The potential allergenicity of all proteins encoded by genes that are introduced into plants, regardless of source, should be assessed before market introduction of the new plant variety. In cases where genes are obtained from allergenic sources, such as peanuts, it should be assumed that the gene encodes an allergen unless clear scientific evidence indicates that it does not. In cases where allergens or suspected allergens are transferred into plants, consumers must be informed of the existence of the allergen in a food by the use of appropriate labelling. In fact, the US Food and Drug Administration (FDA) has adopted a policy that reflects these principles³². Subsequently, a comprehensive science-based approach to assess the allergenicity of foods derived from transgenic plants that best accounts for these principles has been developed¹⁴.

Proteins encoded by genes from allergenic sources: A special case

As already mentioned, if a gene that is transferred into a food crop is obtained from a source that is known to be allergenic, data should be generated to assure that the gene does not encode an allergen³². For this part of an assessment, a layered or tiered approach is used whereby each subsequent level of evaluation represents a greater degree of certainty that the source protein is not an allergen (Fig. 2).

The first level employs *in vitro* assays such as radio-allergosorbent tests, ELISAs or immunoblotting assays

to detect the presence of one or more allergens in the transgenic plant or as a purified gene product. Such assays should use IgE fractions of pooled serum from individuals who are actively sensitive (allergic) to the food from which the transferred gene was derived. Serum donors should meet clinically relevant criteria including a convincing history and/or positive responses in DBPCFCs^{4,9}. Data from one or more of these *in vitro* assays provide strong evidence of the allergenicity or non-allergenicity of a given gene product. A clear positive result from such *in vitro* tests would require that any food containing the introduced gene product be labelled as containing a protein derived from that source.

If no differential response is observed during *in vitro* testing, experiments proceed to *in vivo* skin prick testing. A positive result from this *in vivo* test would raise the same concerns as the *in vitro* tests discussed above.

If the protein does not elicit a positive response in either of these two types of tests, a final evaluation could be performed that involves DBPCFCs with sensitive and non-sensitive individuals under controlled clinical conditions. Those individuals who were known to be allergic to proteins from the source organism would be tested directly for hypersensitivity to food containing the protein encoded by the gene from the allergenic source. Food from non-transgenic lines would be used as a negative control. Finally, if a reaction was provoked in sensitive individuals, food derived from crops containing the protein would require appropriate labelling. If adverse reactions were not observed, it could be concluded that the gene obtained from the allergenic source did not encode one of the endogenous allergens, and therefore the product could be marketed without labelling.

Recently, a gene from the Brazil nut was purposely introduced into soybean to enhance its nutritional quality. The allergy assessment outlined above revealed that the Brazil-nut gene encoded a major Brazil-nut allergen³³. This case provides proof of the effectiveness of *in vitro* and *in vivo* assays to detect allergens that have been transferred into transgenic plants. Consequently, the transgenic soybeans containing the Brazil-nut allergen will not be marketed³³.

In cases where suitable immunological reagents are not available, then the introduced protein should be assessed in the same manner as proteins from non-allergenic sources. However, as more recombinant allergens become available, it should be possible to develop highly specific immunodiagnostic reagents, as described above, which could easily be used instead of IgE serum in the *in vitro* testing procedure. For example, a number of antibodies developed against different recombinant food allergens could be combined into a single diagnostic procedure capable of detecting all the presently known major food allergens. In addition, immunologic reagents based on mouse or rabbit antibodies rather than human serum would be inherently safer to use in practice, because rabbit and mouse sera are not likely to contain human blood-borne pathogens such as hepatitis and HIV.

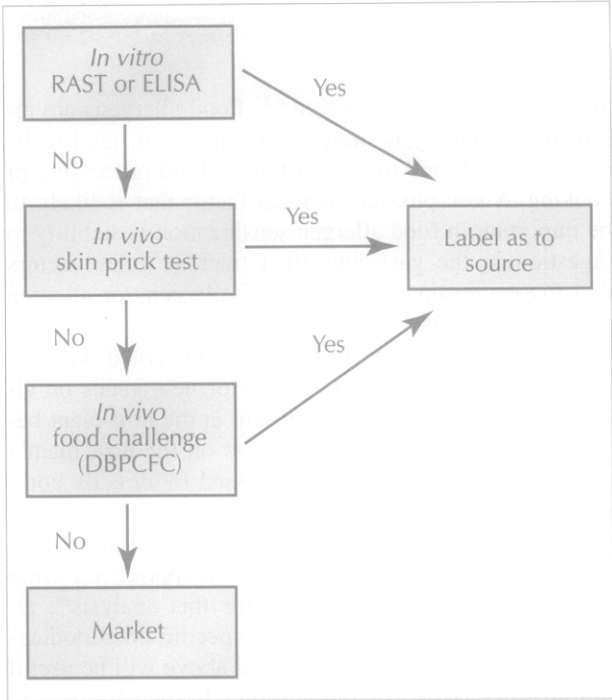


Fig. 2
Assessment of the allergenicity of proteins obtained from allergenic sources. RAST, radioallergosorbent test; ELISA, enzyme-linked immunosorbent assay; DBPCFC, double-blind placebo-controlled food challenge.

Proteins encoded by genes from non-allergenic sources
Evaluating whether a protein from a non-allergenic source has the potential to cause allergy is challenging because there is no single method to predict whether a protein will have the capacity to sensitize naive individuals^{14,34,35}. Therefore, a variety of factors should be evaluated when considering the potential allergenicity of proteins introduced into plants from non-allergenic sources, as shown in Box 1.

Two major factors are used to assess the potential allergenicity of a protein. First, when a candidate gene is being considered for transfer into a plant, the gene is usually well characterized – both the DNA and amino acid sequences are known. As a starting point, a search for amino acid sequence homology of the introduced protein with known allergens should be performed.

Second, an analysis of the physicochemical properties of the introduced protein should be conducted. The main physicochemical characteristics of common food

Box 1. Factors to be considered in an allergy assessment of food derived from transgenic plants

- Source of gene (is it derived from an allergenic food)
- Sequence similarity to known allergens
- Physicochemical parameters:
 - Stability to digestion
 - Prevalence of expressed protein in food
 - Stability to food processing and/or cooking
 - Sequence similarity to non-allergenic proteins
- Molecular genetic effects on the host plant

allergens have been reviewed^{36,37}. Food allergens are resistant to proteolysis, may be glycosylated, tend to be abundant, and tend to be resistant to food processing or cooking. A key physicochemical factor that is likely to be important in food allergen sensitization is stability to digestion in the gastrointestinal tract, or other factors that favor intestinal absorption of allergens or allergen peptide fragments.

The last factor listed in Box 1 is concerned with the possible molecular genetic effects of new genes on allergens that may already be present in the host plant before their introduction. Such effects on the host plant's endogenous allergens can be assessed by directly comparing the levels of known allergens in the non-transgenic parental varieties with the levels in the new transgenic variety of the plant. Immunoblot approaches using IgE antibodies have been used for this analysis³⁸, although it is anticipated that more specific immunodiagnostic tests such as those described above will be useful in this type of analysis in the future. In the case of soybean, which naturally contains allergens, the process of introducing genes that conferred tolerance to the Roundup® (a trademark of Monsanto) herbicide did not alter the levels of these endogenous allergens³⁸. Indeed, it is anticipated that molecular genetic effects will be rare.

Bioinformatics for biotechnology traits

The optimal peptide length for binding to occur between antigens and antibodies is between 8 and 12 amino acids³⁹. In addition, exact conservation of epitope sequences is often observed in homologous allergens from disparate species⁴⁰. However, the situation is more complex and less well defined for conformational epitopes; thus, sequence-based comparisons should be used only as indicators of potential linear epitopes.

Although it is presently difficult to account for the molecular and structural requirements of allergenic epitopes, a sequence comparison test for similarity between the amino acid sequence of the introduced protein and known allergens has been developed: a significant sequence similarity requires an identical match of at least eight contiguous amino acids^{41,42}. It is recognized that exceptions to this rule are likely to occur, but in the absence of specific epitope information, this test provides an estimate of whether there is a strong theoretical possibility that the introduced protein may share an epitope with a known allergen.

A computer program such as FASTA⁴³ (available over the Internet; see below), and the test criteria mentioned above, can be used to test for homology and immunologically relevant sequence similarity between proteins introduced into plants and allergens retrieved from the databases. The lack of any significant amino acid sequence homology enables the conclusions that the introduced gene does not encode a known allergen or an evolutionary related (homologous) protein, and that the introduced gene does not encode an immunologically relevant epitope to be reached. If significant amino acid sequence similarity is observed, there is a

possibility that the transferred protein may be allergenic. Therefore, the gene should be treated as though it was obtained from an allergenic source, as described above.

Allergens are stable in a food digestion model

In addition to comparing amino acid sequences, assessing the digestive stability of proteins is an important indicator of allergenic potential. The ability of food allergens to reach and to cross the mucosal membrane of the intestine is a likely prerequisite to allergenicity. Clearly, a protein that is resistant to the proteolytic enzymes and acidic conditions in the stomach has an increased probability of reaching the intestinal mucosa.

A simulated mammalian gastric digestion system has been used to compare systematically the relative stability to digestion of several common food allergens with that of proteins engineered into plants. Eighteen common food allergens, including the major egg, milk, peanut, soybean and mustard allergens, have been shown to be differentially stable to digestion in simulated gastric fluid. In contrast, common non-allergenic plant proteins were rapidly digested in the gastric model^{41,42,44}.

The demonstration that proteins expressed in transgenic plants undergo rapid degradation greatly reduces the likelihood that they might function as sensitizing agents that elicit an allergic response.

Prevalence, processing characteristics and sequence similarity to food proteins

Many food allergens are present as major protein components in a specific food, typically ranging from 1% to 80% of the total protein content^{3,36}. In contrast, most proteins encoded by genes that have been transferred into plants are expressed at levels ranging from approximately <0.001% to 0.03% of the raw product on a fresh weight basis, or from <0.01% to 0.4% of the total protein content^{41,42}.

The stability of a protein to various food processing activities may also be important in assessing its allergenic potential. For example, if a protein was engineered into fresh market products such as tomato, squash or lettuce, then processing stability is irrelevant because the product will be consumed fresh. If, on the other hand, a protein was engineered into soybean, wheat or rice, because each of these products will be processed by one or more methods before consumption, the stability of the protein to processing conditions should be taken into account. Box 2 lists foods that are known to retain allergenicity after cooking or processing. Furthermore, if the products used for human consumption do not contain protein (e.g. oils or carbohydrates), consumption would be insignificant, and thus the allergenic potential of the expressed protein greatly minimized or even eliminated.

The final component used in the assessment of the allergenic potential of a newly expressed protein is its amino acid sequence similarity to non-allergenic food proteins. If the introduced protein is highly homologous to a protein that has been safely consumed in existing food, then it is unlikely that the introduced protein is

Box 2. Foods that contain allergens that are resistant to processing or cooking^a

Plant products: peanut, soybean, mustard, hazel nut, almond, wheat, rice, celery, tomato

Animal products: cod fish, shrimp, egg, milk

^aBased on a review by Vieths *et al.*³⁷

allergenic. Most of the proteins that have been introduced into plants show biologically relevant amino acid sequence homology to proteins that are already present in the food supply.

All of the assessment components discussed above must be used to reach a complete decision on the allergenic potential of a protein encoded by a gene from a non-allergenic source.

Animal models for predicting allergenicity

Owing to the variable responses from animal to animal, species to species, and within individuals, animal models are not well suited to predicting the allergenicity of new proteins; however, animal models do have a significant role in understanding the etiology of established allergy^{34,35}. Therefore, animal models are unlikely to be of benefit (at least at the present time) in an assessment scheme intended to prevent the proliferation of allergens or the development of new allergies to new proteins. Similarly, it is unlikely that animal models will serve as tools to identify as yet unknown allergens or cross-contaminating allergens.

In fact, in one example in which an animal model was used to predict allergenicity, it failed to detect the presence of a potent allergen³³. This was the case when the Brazil-nut allergen discussed above was assessed by passive cutaneous anaphylaxis in mice⁴⁵. This study reached the erroneous conclusion that the Brazil-nut 2S albumin gene was a strong candidate for genetic engineering into crop plants to enhance the nutritional quality of foods derived from it. Therefore, it is important that a robust animal model be developed, evaluated and subjected to vigorous validation before implementation in an allergy assessment program.

Hypoallergenic foods

Recombinant DNA techniques provide a unique opportunity to reduce the levels of specific allergens in the food supply. By introducing genes in the antisense orientation (the opposite orientation to that required to produce a protein), the levels of protein produced by the 'sense' orientation can be dramatically reduced. This approach has been used to reduce the levels of the primary allergen in rice significantly. Matsuda *et al.*⁴⁶ cloned the gene encoding the 16-kDa allergenic protein from rice grain, and introduced the gene encoding this protein back into rice in the antisense orientation. The levels of the 16-kDa protein were significantly reduced in rice grain. Further studies are underway to achieve even

Box 3. Useful World Wide Web Internet sites

Allergy and Asthma Network:

<http://www.podi.com/health/aaanma/>

American Academy of Allergy Asthma and Immunology:

<http://execpc.com/~edi/aaaai.html>

FDA Center for Food Safety and Applied Nutrition:

<http://vm.cfsan.fda.gov/index.html>

The Food Allergy Network:

<http://www.foodallergy.org>

International Food Information Council:

<http://ificinfo.health.org/index.htm>

National Agricultural Library – Agricultural Genome Information Server (USDA):

<http://probe.nalusda.gov:8000/>

National Center for Biotechnology Information (National Institutes of Health):

<http://www.ncbi.nlm.nih.gov/>

National Research Council of Canada Hotlink to Biotechnology Regulatory Agencies in North American and Europe (EPA, USDA, FDA, AgCAN, H&WCAN, EnvCAN, ECC):

<http://www.ibr.nrc.ca/ibr/regulations.html>

University of Texas Southwestern Medical Center, Division of Allergy and Immunology – Allergy Resources:

http://www.swmed.edu/home_pages/allergy/alg_list.htm

greater reductions in the levels of this allergenic protein. Other technologies including homologous recombination and gene replacement, which effectively exchange undesirable traits for desirable ones⁴⁷, could be used in other food crops such as peanut and soybean to reduce selectively the levels of specific allergens, although the presence of multiple allergens and multigene families in these food crops makes this prospect more complex.

The Internet (World Wide Web)

Most scientists have been using the Internet in one form or another for more than a decade, but it is only recently that the Internet has become a useful research and information tool for anyone. The Internet represents the one medium where recombinant DNA technologies, bioinformatics and information about clinical allergy become integrated; the free flow of information concerning food allergies is now occurring through various Web servers. Box 3 lists some useful Internet sites, notably the National Center for Biotechnology Information, which can be used to access directly public domain genetic databases such as those mentioned earlier in this article, and from where it is possible to retrieve relevant molecular information about characterized allergens. It is even possible to use a version of FASTA known as

BLASTA by pointing your Web browser to this site, which is sponsored by the US National Institutes of Health. The connectivity provided by the World Wide Web should make it possible to access information that is important for food safety and food allergy research quickly and efficiently, which in turn may play a practical role in the prevention of food allergies.

Conclusions

The robust power of biotechnology, bioinformatics, immunodiagnosics and the Internet will be key technologies in a growing armament available to those involved in the development of public health policy initiatives that are intended to assure the safety of the food supply. Only through the development of well-defined, science-based public health policies and due vigilance and awareness of important emerging technologies will it be possible to decrease the incidence of food allergy and fatal anaphylaxis triggered by food allergens.

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References

- Metcalfe, D.D. (1992) 'The Nature and Mechanisms of Food Allergies and Related Diseases' in *Food Technol.* 46, 136–144
- Sampson, H.A. (1992) 'Food Hypersensitivity: Manifestations, Diagnosis, and Natural History' in *Food Technol.* 46, 141–144
- Sampson, H.A. and Metcalfe, D.D. (1992) 'Food Allergies' in *J. Am. Med. Assoc.* 268, 2840–2844
- Bock, S.A. et al. (1988) 'Double-blind, Placebo-controlled Food Challenges (DBPCFC) as an Office Procedure: A Manual' in *J. Allergy Clin. Immunol.* 82, 986–997
- Wüthrich, B. (1996) 'Epidemiology of Allergies and Intolerances Caused by Foods and Food Additives: The Problem of Data Validity' in *Food Allergies and Intolerances* (Eisenbrand, G. et al., eds), pp. 31–39, VCH
- Hefle, S., Nordlee, J. and Taylor, S. 'Allergenic Foods' in *Allergenicity of Foods Produced by Genetic Modification* (Fuchs, R.L. et al., eds), CRC Press (in press)
- Matsuda, T. and Nakamura, R. (1993) 'Molecular Structure and Immunological Properties of Food Allergens' in *Trends Food Sci. Technol.* 4, 289–293
- Panhuysen, C.I.M., Meyers, D.A., Postma, D.S. and Bleecker, E.R. (1995) 'The Genetics of Asthma and Atopy' in *Allergy* 50, 863–869
- Bruijnzeel-Koomen, C. et al. (1995) 'Adverse Reactions to Food' in *Allergy* 50, 623–635
- Valenta, R. and Kraft, D. (1996) 'Type I Allergic Reactions to Plant-derived Food: Consequence of Primary Sensitization to Pollen Allergens' in *J. Allergy Clin. Immunol.* 97, 893–895
- Björkstén, B. (1994) 'Risk Factors in Early Childhood for the Development of Atopic Diseases' in *Allergy* 49, 400–407
- Scheiner, O. and Kraft, D. (1995) 'Basic and Practical Aspects of Recombinant Allergens' in *Allergy* 50, 384–391
- Astwood, J.D. and Hill, R.D. (1996) 'Molecular Biology of Male Gamete Development in Plants – An Overview' in *Pollen Biotechnology: Gene Expression and Allergen Characterization* (Mohapatra, S.S. and Knox, R.B., eds), pp. 1–37, Chapman & Hall
- Metcalfe, D.D. et al. 'Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants' in *Allergenicity of Foods Produced by Genetic Modification* (Fuchs, R.L. et al., eds), CRC Press (in press)
- Scheiner, O. (1992) 'Recombinant Allergens: Biological, Immunological and Practical Aspects' in *Int. Arch. Allergy Immunol.* 98, 93–96
- Sampson, H.A. (1992) 'Food Allergy and the Role of Immunotherapy' in *J. Allergy Clin. Immunol.* 90, 151–152
- Oppenheimer, J.J., Nelson, H.S., Bock, S.A., Christensen, F. and Leung, D.Y.M. (1992) 'Treatment of Peanut Allergy with Rush Immunotherapy' in *J. Allergy Clin. Immunol.* 90, 256–262
- Nakagawa, T. and Gershwin, M.E. (1993) 'Immunotherapy of Allergic Diseases' in *Int. Arch. Allergy Appl. Immunol.* 102, 117–120
- Shimojo, N. et al. (1994) 'Identification of the Disease-related T cell Epitope of Ovalbumin and Epitope-targeted T Cell Inactivation in Egg Allergy' in *Int. Arch. Allergy Appl. Immunol.* 105, 155–161
- Bock, S.A. and Atkins, F.M. (1989) 'The Natural History of Peanut Allergy' in *J. Allergy Clin. Immunol.* 83, 900–904
- Gern, J.E., Yang, E., Evard, H.M. and Sampson, H.A. (1991) 'Allergic Reactions to Milk-contaminated "Nondairy" Products' in *New Engl. J. Med.* 324, 976–979
- Jones, R.T., Squillace, D.L. and Yunginger, J.W. (1992) 'Anaphylaxis in a Milk-allergic Child After Ingestion of Milk-contaminated Kosher-pareve-labeled "Dairy-free" Desert' in *Ann. Allergy* 68, 223–227
- Sampson, H.A., Mendelson, L. and Rosen, J.P. (1992) 'Fatal and Near-fatal Anaphylactic Reactions to Food in Children and Adolescents' in *New Engl. J. Med.* 327, 380–384
- Nordlee, J.A. and Taylor, S.L. (1995) 'Immunological Analysis of Food Allergens and Other Food Proteins' in *Food Technol.* 49, 129–132
- Larkin, M. (1996) 'Immunoassay Usage Increases in Ag and Food Safety Sectors' in *Gen. Eng. News* 16, 1
- Horsch, R.B. et al. (1984) 'Inheritance of Functional Foreign Genes in Plants' in *Science* 223, 496–498
- McHughen, A. and Holm, F.A. (1995) 'Transgenic Flax with Environmentally and Agronomically Sustainable Attributes' in *Transgenic Res.* 4, 3–11
- Padgett, S.R. et al. (1996) 'New Weed Control Opportunities: Development of Soybeans with a Roundup Ready™ Gene' in *Herbicide-resistant Crops. Agricultural, Environmental, Economic, Regulatory, and Technical Aspects* (Duke, S.O., ed.), pp. 53–84, CRC Lewis Publishers
- Falco, S.C. et al. (1995) 'Transgenic Canola and Soybean Seeds with Increased Lysine' in *Biotechnology* 13, 577–582
- Ma, J.K.-C. and Hein, M.B. (1995) 'Immunotherapeutic Potential of Antibodies Produced in Plants' in *Trends Biotechnol.* 13, 522–527
- Poirier, Y., Nawrath, C. and Somerville, C. (1995) 'Production of Polyhydroxyalkanoates, a Family of Biodegradable Plastics and Elastomers, in Bacteria and Plants' in *Biotechnology* 13, 142–150
- US Food and Drug Administration (FDA), Department of Health and Human Services (1992) 'Statement of Policy: Foods Derived from New Plant Varieties' in *Fed. Regist.* 57, 22984–23005
- Nordlee, J.A., Taylor, S.L., Townsend, J.A., Thomas, L.A. and Bush, R.K. (1996) 'Identification of a Brazil-nut Allergen in Transgenic Soybeans' in *New Engl. J. Med.* 334, 688–692
- US Food and Drug Administration, US Environmental Protection Agency, and US Department of Agriculture (1994) *Conference on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops*, April 18–19, 1994, pp. 1–436, Free State Reporting, Annapolis, MD, USA
- Anon. (1996) 'Main Conclusions and Recommendations' in *Food Allergies and Intolerances* (Eisenbrand, G. et al., eds), pp. 223–230, VCH
- Taylor, S.L. (1992) 'Chemistry and Detection of Food Allergens' in *Food Technol.* 46, 146–152
- Vieths, S., Aulepp, H., Becker, W.-M. and Buschmann, L. (1996) 'Characterization of Labile and Stable Allergens in Foods of Plant Origin' in *Food Allergies and Intolerances* (Eisenbrand, G. et al., eds), pp. 130–149, VCH
- Burks, A.W. and Fuchs, R.L. (1995) 'Assessment of the Endogenous Allergens in Glyphosate-tolerant and Commercial Soybean Varieties' in *J. Allergy Clin. Immunol.* 96, 1008–1010
- Rothbard, J.B. and Geffer, M.L. (1991) 'Interactions Between Immunogenic Peptides and MHC Proteins' in *Annu. Rev. Immunol.* 9, 527–565
- Astwood, J.D., Mohapatra, S.S., Ni, H. and Hill, R.D. (1995) 'Pollen Allergen Homologues in Barley and Other Crop Species' in *Clin. Exp. Allergy* 25, 66–72
- Fuchs, R.L. and Astwood, J.D. (1996) 'Allergenicity Assessment of Foods Derived from Genetically Modified Plants' in *Food Technol.* 50, 83–88
- Astwood, J.D. and Fuchs, R.L. 'Allergenicity of Foods Derived from Transgenic Plants' in *Monographs in Allergy Vol. 32: Highlights in Food Allergy* (Wüthrich, B., ed.), Karger (in press)
- Pearson, W. and Lipman, D. (1988) 'Improved Tools for Biological Sequence Comparison' in *Proc. Natl. Acad. Sci. U. S. A.* 85, 2444–2448
- Astwood, J.D. and Fuchs, R.L. (1996) 'Food Allergens are Stable to Digestion in a Simple Model of the Gastrointestinal Tract' in *J. Allergy Clin. Immunol.* 97, 241
- Melo, V.M.M., Xavier-Filho, J., Lima, M.S. and Prouvost-Danon, A. (1994) 'Allergenicity and Tolerance to Proteins from Brazil Nut (*Bertholletia excelsa* HBK)' in *Food Agric. Immunol.* 6, 185–195
- Matsuda, T. et al. (1996) 'Allergenic Proteins in Rice: Strategies for Reduction and Evaluation' in *Food Allergies and Intolerances* (Eisenbrand, G. et al., eds), pp. 161–169, VCH
- Morton, R. and Hooykaas, P.J.J. (1995) 'Gene Replacement' in *Mol. Breed.* 1, 123–132