Introduction: What Are the Issues in Addressing the Allergenic Potential of Genetically Modified Foods?

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There is growing concern among the general public and the scientific community regarding the potential toxicity of genetically modified organisms (GMOs). The use of biotechnology to enhance pest resistance or nutritional value has raised a number of fundamental questions including the consequences of insertion of reporter genes, the spread of resistance genes to surrounding plants, and the use of suicide genes to prohibit reuse of seed from engineered plants. Of particular interest is the ability of proteins from GMOs to elicit potentially harmful immunologic responses, including allergic hypersensitivity. The lack of information of the potential toxicity of these products suggests a need to identify the critical issues and research needs regarding these materials and to develop testing strategies to examine the allergenicity of these compounds. Key words: biotechnology, decision tree, food allergy, genetically modified crops, IgE, immunology, sensitization. Environ Health Perspect 111:1110–1113 (2003). doi:10.1289/ehp.5810 available via http://dx.doi.org/[Online 19 December 2002]

Evaluating the Allergenic Potential of Genetically Modified Foods

The application of biotechnology to food production offers great promise in increasing crop production through development of plants that have an increased yield through resistance to changes of temperature and drought and the expression of natural pesticides, lessening the need for the application of exogenous pesticides (Astwood et al. 1997; Mann 1997). Plants may also be developed in which toxin content is downregulated, immunizing proteins are expressed, fat/protein ratio is altered, palatability is increased, and appearance is more appealing (Arntzen 1998). Crops may be developed that naturally express vitamins or that are deficient in specific allergens known to cause problems upon ingestion by individuals sensitive to the allergens within the native plant (Friedrich 1999).

It is inevitable that application of this technology has raised a number of fundamental concerns, including the consequences of reporter genes, the spread of resistance genes to surrounding plants, the use of suicide genes to prohibit reuse of seed from engineered plants, and finally, whether these altered plants may be allergic (Lehre et al. 1996; Miller 1993; Taylor 1997; Van Dam and de Vriend 1999; Wal and Pascal 1998). It is this last question that was the subject of the conference “Assessment of the Allergic Potential of Genetically Modified Foods” held 10–12 December 2001 in Research Triangle Park, North Carolina. Specifically, how do we determine if a genetically modified food is likely to be allergenic, given that peanut and tree nut allergies alone are observed in 1.1% of the general U.S. population (Sicherer et al. 1999)?

Overview of Allergic Reactions to Foods

To address this question, it is first necessary to appreciate that a number of defined clinical pathologic entities fall within the general public perception of what defines food allergy (Table 1) (Burks et al. 1988; Lake 1997; Min and Metcalfe 1997; Smith and Munoz-Furlong 1997; Solid 1989). Indeed, the majority of reactions to foods are classic allergic reactions (Jansen et al. 1994). These reactions occur within minutes after ingestion of a food and are manifested by urticaria, angioedema, rhinoconjunctivitis, nausea, vomiting, diarrhea, and asthma. These reactions depend on the synthesis of antigen-specific immunoglobulin E (IgE) by B cells in allergic individuals with an inherited tendency for T-helper type 2 cell–like reactions, in which the T cells, when activated, secrete cytokines such as interleukin (IL)-4 and IL-5. This antigen-specific IgE becomes fixed on the surfaces of mast cells and basophils within that sensitized individual. Basophils circulate through the blood, and mast cells are found with greater frequency on surfaces of the body that interface the external environment. When that individual is reexposed to the same antigen, it then cross-links IgE on the surfaces of basophils and mast cells. This leads to basophil and mast cell activation, with the release of histamine, generation of arachidonic metabolites, and the release and generation of potent cytokines and chemokines. These chemicals then interact with target-sensitive tissues and generate the allergic response. For instance, if mast cells are activated within the gastrointestinal tract, this then leads to nausea, vomiting, diarrhea, and the ingress of antigen into the systemic circulation where it may degranulate basophils within the vascular compartment and mast cells within target tissues. If such a reaction is severe, it may result in profound hypotension and be life threatening. This latter reaction is called anaphylaxis.

However, not all reactions to foods on an immunologic basis are IgE mediated. There are non–IgE-mediated delayed reactions, particularly in infants and children, to such substances as milk protein. These reactions may lead to vomiting, diarrhea, and failure to thrive. Such reactions are sometimes termed “food-induced enterocolitis syndromes” (Lake 1997). The food components that induce such reactions and the mechanisms behind these reactions, which include immune complex formation and activation of lymphocytes, have yet to be fully defined. Similarly, celiac disease (Solid et al. 1989) is a specific form of food allergy in which the body reacts to components of specific cereal grains, termed “glutens,” which leads to a specific pathologic picture.

Efforts to date to address issues of allergenicity in engineered foods have concentrated on IgE-mediated immediate reactions. Not only are these reactions the most frequent, but they are easily the most potentially life threatening. Further, the specific antigens within food that lead to such reactions and the effectors cells and mediators involved have been reasonably well characterized. The same cannot be said for delayed reactions to food components, seen primarily in infants and children. Because gluten-sensitive entropy, or celiac disease, is specifically caused by glutens, any consideration of allergenicity in a new food should specifically address whether genes coding for glutens have been transferred, and thus create products that will be a problem for those with celiac disease. This disease aside, however, the majority of current efforts remain directed at IgE-mediated allergic reactions.

The tools to aid in the diagnosis of food allergy are limited (Bock et al. 1988; Jansen et al. 1994; Pastorello et al. 1989). The most
critical feature in the diagnosis of food allergy is a carefully obtained clinical history of specific reactions to foods. What foods were ingested, in what quantities, and in what context? A careful history often reveals the food that induced the allergic response. The history may be supplemented by careful use of diet diaries and elimination diets (when safe for a given individual). The suspicion that a specific food induced an allergic response may be reinforced by the demonstration of IgE specific to the food in question. Testing usually takes one of several forms. The most common test employed is the use of water-soluble extracts applied to the skin. The skin is then “tented” through the extract. If a person is sensitive to allergens within the food in question, a local, small allergic reaction consisting of erythema, edema, and itching will occur. Alternatively, blood obtained from patients with suspected food allergies can be subjected to examination using a radioallergosorbent test (RAST) or enzyme-linked immunosorbent assay (ELISA), which identifies the presence of food-specific IgE within serum. If history, cutaneous testing, and RAST/ELISA do not demonstrate sensitivity, it may on some occasions be necessary to challenge the patient in a double-blind fashion with suspected foods, masked either in capsules or in another food (Jansen et al. 1994). This latter testing strategy should not be used if the person is anaphylactically sensitive to the suspected food. Further, if such testing is performed, it should be done only by a physician skilled in application of this procedure, in the context of the equipment available for intubation and the treatment of a severe allergic reaction, and with the patient’s informed consent.

Much of the need to address the safety of modified foods is because currently there is no available means to cure a given individual of an immediate reaction to food. The strategy for protection of a sensitive individual involves instruction on dietary avoidance of the foods in question. Patients also are instructed on how to self-treat in the case that inadvertent exposure occurs. Immediate treatment for systemic reactions is the intramuscular injection of epinephrine followed by acquisition of medical help. The reliance on dietary avoidance by patients with food allergies is the reason it is so important that new allergenic foods not be created. The difficulty in this strategy is that the characteristics of a protein with known allergenicity that would distinguish this protein from a protein unlikely to be allergenic are not known. This in turn dictates that strategies to screen for allergenicity must rely on what is known or what can be determined about proteins coded for by transferred genes or upregulated within the target plant. To address these concerns, a series of questions can be asked similar to those in Table 2. Does the gene transferred from a source material to the altered plant code for a known allergen? Two approaches in this regard have been widely applied. First, is there sequence and structural similarity between the transferred gene product and known allergens? Second, does the transferred gene code for a protein known to be allergenic? This is particularly relevant when the gene is derived from a known allergenic source. In this latter case, serum from individuals sensitive to the source of the transferred gene can be used to screen for the presence of IgE reactive to the transferred protein as expressed in the modified food. Clearly, simply examining sequence similarity does not account for discontinuous and conformational epitopes. However, with crystallization of known allergens, it is possible to foresee a day when such epitopes may be identified. A more difficult question is whether the transferred gene codes for a protein that may be allergic, when the source of the transferred protein is not known for its allergenicity. Again, efforts can be made to determine sequence similarity of the transferred gene to known genes that code for allergens. Here, resistance to degradation by proteases and acid has been suggested as a relative screening methodology (Attrwood et al. 1996). Resistance of a transferred protein to degradation partly based on the theory that resistance to degradation protects a protein from digestion and thus allows for greater absorption of the protein. Assessment of transferred proteins for potential allergenicity has also generated interest in the possible use of animal models to assess protein allergenicity. This leads to another question. Has the genetic manipulation of the modified food upregulated endogenous substances that may be allergenic? In the possible instance where such upregulated proteins may not have been identified, screening for allergenicity again through the use of an animal model has been attractive.

### Decision Tree Approaches

The first organized attempt to synthesize a plan in the form of a decision tree to address issues of the allergenicity of engineered foods was sponsored by the International Food Biotechnology Counsel and the International Life Sciences Institute. This decision tree and accompanying articles were published in a supplement of Critical Reviews in Food Science and Nutrition (Metcalfe et al. 1996) after a number of other similar initiatives (FAO/WHO 1995, 1996; U.S. FDA 1992). This decision tree envisioned two primary scenarios. The first scenario is that the source of the transferred gene was allergenic. In this case, serum from individuals allergic to the source of the gene could be used to screen extracts of the modified food to determine if an allergenic protein had been transferred. The initial screening is done using an in vitro measurement of allergen-specific IgE, possibly followed by skin testing of extracts of the modified plant in individuals known to be sensitive to the source material, and finally, by the rare possibility of employing blinded food challenge procedures in sensitive individuals. In the second scenario, where the source of

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**Table 1. Clinical patterns of immune-mediated adverse reaction to foods.**

| IgE-mediated (immediate) | Urticaria/angioedema | Rhinocconjunctivitis, asthma | Oral allergy syndrome | Nausea, colic, vomiting, diarrhea | Anaphylaxis |
|--------------------------|----------------------|------------------------------|----------------------|---------------------------------|------------|
| Food-induced enterocolitic syndromes | Celiac disease | Overlap | Atopic dermatitis | Allergic eosinophilic gastroenteritis | Controversial | Migraine headaches | Irritable bowel syndrome, etc. |

**Table 2. Examples of technical approaches to address questions concerning the possible allergenicity of modified foods.**

| Questions | Sequence similarity | Reaction to specific IgE | Stability to digestion | Allergenicity in an animal model |
|-----------|---------------------|--------------------------|------------------------|------------------------|
| Does the gene transferred code for a known allergen? (Source is a common allergenic food.) | X | X | X |
| Does the gene transferred code for a protein that may be allergenic? | X | X | X |
| Does the genetic manipulation lead to the expression or upregulation of a new allergen? | X |
the gene is not known to be allergenic, the decision tree recommends examining the transferred gene for sequence similarity to known allergens and for stability to digestion. This decision tree has been widely discussed and critiqued.

This initial decision tree was published in 1996 at a time when a number of modified foods were in the process of being approved. However, with the increasing use of genetically modified plants in the late 1990s came a significant rise in public concern about the safety of genetically modified foods (Enserink 1999; Ferber 1999; Gaskell et al. 1999). Among the many issues of public concern was the issue of allergenicity. In part, public attention to these issues was heightened by the circumstances surrounding the approval of StarLink corn. In 1998, the U.S. Environmental Protection Agency (U.S. EPA) decided to limit the use of this corn for animal feed because the Cry9C protein engineered into this corn to provide pesticide resistance was resistant to degradation. Subsequently, in September 2000, Cry9C DNA was detected in taco shells, and the U.S. EPA and U.S. FDA became involved in an assessment of whether the StarLink corn was indeed allergenic (U.S. EPA 2001).

Over the same period, a number of criticisms had been voiced about the 1996 International Life Sciences Institute/International Food Biotechnology Consortium (ILSI/IFBC) decision tree approach in assessing allergenicity. In some cases, the use of skin prick tests and double-blind placebo-controlled food challenge were felt to be inappropriate because of ethical concerns in the use of normal volunteers for the purposes of safety assessment. There was also significant concern over exactly how many contiguous amino acids defined sequence similarity. There was increasing evidence that food digestion was a poor screen for allergenicity. Further, there was no screen for cross-reacting allergens. Finally, individuals interested in animal models of allergenicity were making the case that such models would help to define allergenicity for safety assessment.

To address these concerns, the FAO/WHO convened a special panel in 2001 to update the ILSI/IFBC decision tree. This revised decision tree most noticeably differs from the ILSI/IFBC decision tree through the insertion of screens for cross-reacting allergens such as those that exist between ragweed and melons, and through its advocacy of the use of animal models in a relative scale of allergenicity (FAO/WHO 2001). The FAO/WHO panel also recommended that such approaches to assess allergenicity must constantly take into account new and evolving information on parameters that define allergenicity and on the evolving use of specific animal models. They also noted that use of human in vivo methods to evaluate the allergenicity of food derived from biotechnology in many circumstances raised ethical issues, and their use would have to be considered on a case-by-case basis and as relates to the value of premarketing evaluation. This was particularly important in the assessment of foods claimed to be hypoallergenic through the downregulation of genes that code for known allergens.

Questions in Risk Assessment

If it was possible to list with certainty the characteristics that allow a specific protein to induce specific IgE in a susceptible individual, there would have been no need for this conference. The reality is, however, that we have only imperfect and relative measures of allergenic potential at present (Table 2). Given the current situation, what issues now need to be addressed?

It may be argued that the first set of issues centers around the understanding of pathophysio logic mechanisms of allergenicity. How do we define susceptible populations? What are the thresholds for sensitization? What are the thresholds for elicitation of a reaction? What do we know about dose–response relationships between the amount of food ingested and the final reaction? And finally, what available biomarkers are there of exposure and effect?

A second series of issues related to the mechanisms of allergenicity is directed to allergic structure. What are the molecular determinants of allergenicity? Is there a relationship between allergenicity and function? And finally, is it possible to use animal models to predict allergenicity?

Assuming that any effort to identify genetically modified foods of potential allergenicity using current technology will not be perfect, what then is the role of postmarket surveillance? Is it practical to label genetically modified foods (GMO) foods, considering that many of these foods will be processed? And what about the issue of postmarketing surveillance in situations where foods are sold in restaurants and through street vendors and in other situations where monitoring of labeling becomes difficult?

It was thus the charge of this meeting to examine the issues surrounding the potential allergenicity of GMO foods. What is the true value and role of an assessment of new proteins in GMO foods in terms of similarity to known allergens? How many contiguous amino acids define similarity? What are the limitations and strengths of tests to examine stability of proteins? Is there sufficient information to support the use of animal models in evaluating allergens to allow their rational use in safety assessment? To what degree can safety assessment rely on testing of GMO foods using sera from those known to be allergic to source materials, pollens, and other substances in the environment? In the end, decisions made as to how to apply existing knowledge and databases in the assessment of GMO foods for potential allergenicity will be only as successful as they are creditable to research scientists, industry, and to the public at large.

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