Clinical and Laboratory Investigation of Allergy to Genetically Modified Foods

Jonathan A. Bernstein,[1] I. Leonard Bernstein,[1] Luca Bucchini,[2] Lynn R. Goldman,[2] Robert G. Hamilton,[3] Samuel Lehrer,[4] Carol Rubin,[5] and Hugh A. Sampson[6]

[1]Department of Medicine, University of Cincinnati, Cincinnati, Ohio, USA; [2]Bloomberg School of Public Health and [3]School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA; [4]Department of Medicine, Tulane University Health Sciences Center, New Orleans, Louisiana, USA; [5]Health Studies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [6]Department of Pediatrics, Mt. Sinai School of Medicine, New York, New York, USA

Technology has improved the food supply since the first cultivation of crops. Genetic engineering facilitates the transfer of genes among organisms. Generally, only minute amounts of a specific protein need to be expressed to obtain the desired trait. Food allergy affects only individuals with an abnormal immunologic response to food—6% of children and 1.5–2% of adults in the United States. Not all diseases caused by food allergy are mediated by IgE. A number of expert committees have advised the U.S. government and international organizations on risk assessment for allergenicity of food proteins. These committees have created decision trees largely based on assessment of IgE-mediated food allergenicity. Difficulties include the limited availability of allergen-specific IgE antisera from allergic persons as validated source material, the utility of specific IgE assays, limited characterization of food proteins, cross-reactivity between food and other allergens, and modifications of food proteins by processing. StarLink was a corn variety modified to produce a *Bacillus thuringiensis* (Bt) endotoxin, Cry9C. The Centers for Disease Control and Prevention investigated 51 reports of possible adverse reactions to corn that occurred after the announcement that StarLink, allowed for animal feed, was found in the human food supply. Allergic reactions were not confirmed, but tools for postmarket assessment were limited. Workers in agricultural and food preparation facilities have potential inhalation exposure to plant dusts and flours. In 1999, researchers found that migrant health workers can become sensitized to certain Bt spore extracts after exposure to Bt spraying. Thus, the potential for occupational and consumer risks needs to be assessed. *Key words:* allergens, *Bacillus thuringiensis*, crops, endotoxins, food hypersensitivity, genetic engineering, genetics, immunology, recombinant proteins, transgenic plants. *Environ Health Perspect* 111:1114–1121 (2003). doi:10.1289/ehp.5811 available via *http://dx.doi.org/* [Online 19 December 2002]

Technology has been used for many years to improve our food supply since the first cultivation of crops such as wheat and barley in Mesopotamia in 6000 BC and the domestication of animals such as sheep and goats in southwestern Asia over 10,000 years ago. More recently, improvement of our food supply through genetic manipulation by breeding was accelerated in the development of hybrid crop varieties in the 1960s and 1970s (a period referred to as the green revolution), which more than doubled the crop production in developing countries. Breeding and selection have been used for many domesticated animal species that are food sources. A good example is chicken, one of the more expensive meats in the 1940s, and now one of the least expensive sources of meat. Cultivation, domestication, breeding, and selection of certain traits of plants and animals have created environmental impacts and major changes to human societies. Although use of technology in breeding plants and animals is not new, new methods of biotechnology incorporate genetic engineering, also referred to as molecular breeding. Genetic engineering facilitates the selection, identification, and transfer of genes encoding for a specific protein into the genome of another organism. This process can determine which proteins are introduced and where they are expressed; in most cases, only minute amounts of a protein need to be expressed to obtain the desired trait.

In assessing the public health aspects of genetically engineered foods, it is the proteins that are expressed that are of interest. Three possible modes of adverse health effects have been hypothesized: toxicity, impaired nutrition, and food allergy. Modifications of expression of protein in foods occur with all kinds of plant breeding, and these theoretical concerns are not unique to genetically engineered foods. However, because genetic engineering is a more powerful tool for making such changes, government authorities in the United States, Europe, Japan, Canada, and elsewhere have taken actions to regulate this class of foods.

In this article, we describe various clinical aspects of food allergy relevant to the assessment of novel proteins in genetically modified foods. Food allergy is among a spectrum of adverse reactions that can result when an individual ingests food or a food additive. Evaluation of novel proteins for potential allergenicity is based on a fundamental understanding of the clinical and biological aspects of these responses. Among food allergy researchers, a number of definitions of food allergy have been used. For the purpose of this article, allergy is defined as hypersensitivity and implies an immunologic reaction to food; other (nonimmunologic) adverse reactions to food will be referred to as "intolerance." This reflects the standard definitions of terms in the United States. It is important to note that the revised nomenclature for allergy proposed by the European Academy of Allergy and Clinical Immunology uses the terms "nonallergic food hypersensitivity" instead of "intolerance," and "food allergy" (IgE-mediated or not) instead of "hypersensitivity." Defined in this manner, food allergy affects only those individuals who have developed an abnormal immunologic response to food. To understand the public health impacts of food allergy, we must appreciate both the prevalence in the population and the clinical spectrum of food allergy.

Although there are a number of clinical manifestations of food allergy, most of the focus for clinical evaluation of genetically modified foods has been on IgE-mediated anaphylaxis. Various expert committees, including a committee of the International Life Sciences Institute, an advisory committee to the U.S. Environmental Protection Agency (U.S. EPA), and committees convened by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), have developed decision trees for identifying whether novel proteins cause such anaphylactic reactions. These decision trees are largely based on the clinical and laboratory methods used to assess the potential allergenicity of foods. These methods were developed for clinical purposes but now are being used to predict allergenicity and for postmarket surveillance. The StarLink corn episode provided an opportunity for researchers to assess the utility of the decision tree as well as the ability to conduct postmarket surveillance for allergic reactions in foods in commerce. None of the reported allergic reactions were confirmed, and it is possible that this episode

This article is part of the mini-monograph "Assessment of the Allergenic Potential of Genetically Modified Foods."

Address correspondence to L. Goldman, Bloomberg School of Public Health, Johns Hopkins University, 615 N. Wolfe St., Room 8511, Baltimore, MD 21205 USA. Telephone: (410) 614-9301. Fax: (443) 287-7075. E-mail: lgoldman@jhsph.edu

The authors declare they have no conflict of interest.

Received 31 May 2002; accepted 9 December 2002.

resulted from the media coverage and public perceptions about the risks. This episode demonstrated the limitations of using clinical tools for assessment of food allergy in postmarket assessment. Another clinical issue is the potential for development of inhalation allergy from novel proteins in foods.

Clinical Spectrum of Food Allergy

H. A. Sampson presented the clinical spectrum of adverse reactions to food, including food allergies. Estimates of the prevalence of adverse food reactions vary widely depending on whether they are defined using the "gold standard"—DBPCFC (double-blind placebo-controlled food challenge)—or as reported reports by patients and their families. In one prospective study of children, 6% of children had food allergy or intolerance confirmed by DBPCFC, which is lower than the 28% among children whose parents reported adverse food reactions (Bock 1987). A number of prospective studies indicate that allergy to cow's milk is prevalent among 2.5% of children under the age of 2 years (Bock 1987; Hide and Guyer 1983; Host et al. 1988; Schrander et al. 1993), with an overall prevalence rate at some time during childhood of 6%. Some children are at greater risk; food allergy occurs in 30% of children who have atopic dermatitis (Burks et al. 1988). Food allergy is less common in adults, affecting between 1.5 and 2% of adults in the United States (Jansen et al. 1994; Young et al. 1994).

The development of food allergy most often occurs in early childhood before the age of 3 years. It involves mechanisms related to the nature of the food allergen, the gastrointestinal tract, and the immune system (Table 1) (Sampson 1993). Food allergens are proteins, usually glycoproteins, and they generally share certain physical and chemical characteristics (Table 1). Only a few foods are known to cause the vast majority of allergic reactions: in children—eggs, peanuts, milk, soy, and wheat; in adults—shellfish, fish, nuts, and peanuts. The gastrointestinal tract has a number of nonspecific barriers to the entry of foreign proteins, and one specific barrier, secretory IgA, produced by the immune system (Table 1). Nonetheless, food proteins can be absorbed into the bloodstream and carried to target organs. Mechanisms have evolved to ensure that the immune system does not attack one's own proteins ("self") and proteins in food. The general process by which the immune system is programmed not to attack such proteins is called "tolerance." Tolerance is therefore a barrier to the development of food allergy, and when people become sensitized to food, this is a breakdown of tolerance.

Clinical manifestations. Food allergies are mediated either by IgE or by other immunologic mechanisms. IgE-mediated reactions are the most common in the general population. Non–IgE-mediated food allergies account for a significant proportion of food allergies in infants and young children. As shown in Table 2, food allergy can cause a multitude of clinical manifestations.

IgE-mediated food allergies. IgE-mediated food allergies typically develop in an individual within minutes to hours after ingesting a food allergen. IgE-mediated food allergies can have a longer onset in the case of food that has been ingested more frequently, thus blunting the immediate symptoms and resulting in symptoms of a chronic inflammatory reaction that may last for several days. A number of skin manifestations can occur. On an immediate basis, acute urticaria/angioedema often occurs following the ingestion or contact with a food. Chronic urticaria/angioedema related to food allergy is rare (Champion et al. 1969; Volonakis et al. 1992). Children may develop atopic dermatitis. During blinded food challenges, an itchy, red, morbilliform (measles-like) rash develops within 10 to 90 min of allergen ingestion (Sampson and McCaskill 1985). Repeated ingestion of the allergen may result in the development of an itchy, eczematous rash (Sampson 1992).

Food allergy can cause both upper and lower respiratory symptoms (Bock 1992; James et al. 1994). Recently, James et al. (1996) established that food allergy can provoke increased airway hyperreactivity in food-allergic patients with asthma, and two studies (Novembre et al. 1988; Oehling and Baena Cagnani 1980) demonstrated food-induced wheezing in 6–8% of unselected asthmatic patients.

Symptoms involving the oropharynx and gastrointestinal tract may occur within minutes of ingesting a food allergen. Itching and swelling of the lips, tongue, and soft palate as well as nausea, abdominal pain, vomiting, and diarrhea have all been demonstrated secondary to food allergy. The oral allergy syndrome consists of symptoms confined exclusively to the oropharynx and is most commonly reported in patients with seasonal allergic rhinitis after the ingestion of one of a variety of fresh fruits and vegetables (Ortolani et al. 1988; Pastorello et al. 1994). Gastrointestinal anaphylaxis frequently accompanies symptoms in the skin or respiratory tract and presents as nausea, abdominal cramping, vomiting, and diarrhea. Repeated ingestion of food allergens in young children may induce partial desensitization, resulting in less-obvious symptoms, e.g., gastroesophageal reflux (GER) instead of projectile vomiting

Table 1. Mechanisms of the development of food allergy.

	Characteristics
Food allergens	Proteins, usually glycoproteins, generally believed to be water soluble, heat resistant, acid stable, 10–60 kDa molecular weight
Gastrointestinal tract	Nonspecific barriers Gastric acid and pepsin Pancreatic and intestinal enzymes Mucus secretions Peristaltic activity Mucosal epithelial cells Specific barrier Specific secretory-IgA can block the absorption of foreign proteins
Immune systems	Development of immunologic tolerance

Table 2. Food-allergic illnesses.

	Organ system	Clinical manifestation
IgE-mediated	Skin	Urticaria/angioedema[a] Atopic dermatitis
	Respiratory	Rhinoconjunctivitis Laryngeal edema Asthma
	Gastrointestinal	Nausea and abdominal cramps Vomiting and diarrhea Oral allergy syndrome Infantile colic (rare)
	General	Anaphylactic shock[a]
Non–IgE-mediated	Skin	Dermatitis herpetiformis Contact dermatitis
	Respiratory	Heiner's syndrome
	Gastrointestinal	Food-induced enterocolitis Food-induced eosinophilic proctocolitis Food-induced enteropathy and celiac disease Allergic eosinophilic gastroenteritis Gastroesophageal reflux Infantile colic (rare)

[a]Symptoms also may be provoked by the combination of ingesting specific food(s) in conjunction with exercising but not by ingestion of the food alone or exercise alone.

(Iacono et al. 1996). A minority of patients with infantile colic (inconsolable, agonized crying, drawing up of the legs, abdominal distention, and excessive gas associated with feeding during the first several months of life) have symptoms attributed to IgE-mediated food hypersensitivity (Sampson 1989).

Food-induced systemic anaphylaxis has been reported to be the leading single cause of anaphylaxis in emergency departments in the United States (Kemp et al. 1995; Yocum and Khan 1994). In two reports of fatal anaphylactic reactions (Sampson et al. 1992; Yunginger et al. 1988), the authors noted that all subjects had asthma, had unknowingly ingested the responsible food allergen, and had tended to minimize the symptoms initially, and that the initiation of emergency medical management was delayed. Anaphylactic shock in association with exercise 2–4 hr after the ingestion of certain foods is being recognized increasingly, especially in young women (Horan and Sheffer 1991; Romano et al. 1995).

Non–IgE-mediated allergic reactions. Non–IgE-mediated allergic reactions are believed to take several hours to days to develop, and a variety of disorders have been delineated. A variety of gastrointestinal disorders believed to have an immunologic basis have been described. Food-induced enterocolitis syndrome is seen most frequently in young infants ingesting cow's milk- or soy-based formulas. It generally presents between 1 week and 3 months of age, with protracted diarrhea and projectile vomiting often severe enough to produce dehydration (Powell 1978). The syndrome also is seen in exclusively breast-fed infants (secondary to the passage of food proteins in maternal milk) and occasionally in older children (associated with ingestion of egg, wheat, rice, peanut, nuts, chicken, turkey, and shellfish). Benign eosinophilic proctocolitis, also present in the first few weeks to months of life, is often secondary to cow's milk or soy, although about half the infants are being exclusively breast-fed (Machida et al. 1994; Odze et al. 1995). Patients appear clinically well and present only with bloody stools (gross or occult) or hematochezia. Lesions are confined to the distal bowel and vary from mucosal edema to ulceration and linear erosions. Both enterocolitis and proctocolitis show dramatic clinical resolution within 72 hr of allergen elimination.

Food protein–induced enteropathy includes a spectrum of malabsorption disorders that generally present with protracted diarrhea, vomiting in up to two-thirds of patients, failure to thrive, and carbohydrate malabsorption. Increased fecal fat and abnormal D-xylose absorption generally are present. Cow's milk sensitivity is the most frequent cause of this syndrome, but it also has been associated with soy, egg, wheat, rice, chicken, and fish hypersensitivity. Patchy villous atrophy with cellular infiltrate on biopsy is characteristic of this disorder (Kuitunen et al. 1975; Nagata et al. 1995). A more extensive enteropathy, with total villous atrophy and extensive cellular infiltrate (celiac disease), is associated with sensitivity to gliadin, a component of gluten. These patients often present with diarrhea or frank steatorrhea, abdominal distention and flatulence, weight loss, and occasionally nausea and vomiting.

Dermatitis herpetiformis is a highly itchy skin rash (sometimes mistaken for atopic dermatitis) associated with gluten-sensitive enteropathy (Hall 1987). Biopsy of the skin rash reveals an infiltration of polymorphonuclear leukocytes and deposits of IgA at the dermal-epidermal junction. Administration of dapsone or other sulfones often relieves the skin itching within 24 hr. Like celiac disease, elimination of all gluten for 3–4 months may be required to normalize intestinal biopsy findings.

Allergic eosinophilic gastroenteritis (AEG) may involve food allergy (Min 1991), as may infantile colic. AEG often presents as postprandial nausea with vomiting, abdominal pain, diarrhea, occasionally steatorrhea, and weight loss in the adult, or failure to thrive in the infant (Lee et al. 1993). In the mucosal form, patients often have atopic disease, elevated serum IgE levels, positive immediate skin tests to a variety of foods and aeroallergens, peripheral eosinophilia, iron deficiency anemia, and hypoalbuminemia. Protein-losing enteropathy or pyloric obstruction may be the main feature in some infants with AEG (Snyder et al. 1987; Waldman et al. 1967).

A recent study of 10 patients with AEG and severe GER found that non–IgE-mediated food allergy may be a much more common cause of AEG than previously appreciated (Kelly et al. 1995). Food hypersensitivity is a frequent cause of GER in young infants. Milk allergy was the cause of GER in 85 of 204 (42%) of infants less than 1 year of age (Iacono et al. 1996). Removal of the suspect allergen for up to 12 weeks may be required to bring about resolution of symptoms and intestinal histologic changes.

Natural history of food allergy. Experience and follow-up challenge studies on food-allergic individuals indicate that food allergies are not necessarily lifelong. Studies have demonstrated the loss of food allergy in up to one-third of children (Sampson and Scanlon 1989) in 1–3 years of age, even though results of skin tests and radioallergosorbent assays (RASTs) may not change. Evidence suggests that the likelihood of losing a food allergy is dependent upon the food provoking the symptoms and the degree to which the patient maintains the allergen elimination diet. Allergy to peanut, tree nuts, fish, and other seafood appears to be more long-lasting (Sampson and Scanlon 1989).

Diagnosis. Many subjective complaints have been ascribed to adverse food reactions, including neurologic (dizziness, weakness, headaches, numbness, loss of concentration, depression), gastrointestinal (generalized bloating, abdominal distention, constipation), musculoskeletal (muscle cramps, myalgia, arthralgia, vasculitis), and miscellaneous complaints (sweating, chest pain, fatigue, itchy earlobes) (National Research Council 2000b). Such symptoms can rarely, if ever, be confirmed with blinded food challenges.

Evaluation of a patient for suspected adverse food reactions involves a thorough history, physical examination, and laboratory tests. With the history, an attempt is made to establish whether the patient is suffering from an intolerance or hypersensitivity reaction, and if the latter, whether a non–IgE- or IgE-mediated mechanism is involved. If an IgE-mediated food hypersensitivity is suspected, the clinical impression may be reinforced by performing prick skin tests or *in vitro* diagnostic tests.

Skin testing with food extracts by the prick or puncture method may be helpful. Routine intradermal skin tests with food extracts are too sensitive and nonspecific (leading to excessive false-positive tests), carry a higher risk of provoking systemic reactions, and are not indicated. A positive skin test denotes the presence of allergen-specific IgE antibodies bound to cutaneous mast cells; it does not mean the patient will develop symptoms when ingesting the specific food. In fact, the positive predictive values of most prick skin tests are less than 50%. In contrast, the negative predictive accuracies are excellent, and IgE-mediated allergic reactions are extremely rare in the face of negative skin tests.

In vitro tests of food-specific IgE antibodies are often used in patients with extensive skin disease, significant and prolonged dermatographism, or a history of exquisite sensitivity (i.e., exposure to minute quantities of a specific food resulted in a life-threatening reaction). A newer test, the CAP system FEIA (fluorescent-enzyme immunoassay; Pharmacia Diagnostics, Uppsala, Sweden) quantitates the amount of food-specific IgE antibodies, which correlates better with clinical reactivity.

Elimination diets are used when the history and/or preliminary laboratory studies suggest certain foods may be provoking a patient's symptoms. Foods (and all hidden sources of that food) suspected of inducing symptoms are totally eliminated from the patient's diet for 1–2 weeks. If symptoms appear to improve, further characterization of the sensitivity should be pursued (e.g., endoscopy and biopsy, blinded challenge, and so forth). In several chronic disorders (e.g., atopic dermatitis, asthma, or chronic diarrhea), factors in addition to the food hypersensitivity may be triggering symptoms, so that failure to see resolution of symptoms during the elimination

period does not necessarily rule out food hypersensitivity. In cases where food hypersensitivity or intolerance are suspected but no specific foods can be incriminated, a brief trial (i.e., 2–4 weeks) of an oligoantigenic or elemental diet may be helpful. If patient symptoms persist unabated, it is very unlikely that food is a contributing factor.

In the practice setting, open or single-blind oral food challenges may be used to screen for food allergic reactions. However, in cases where multiple food allergies are diagnosed, positive responses should be confirmed by DBPCFCs. DBPCFCs have been used successfully in both children and adults for examining a variety of food-related complaints. The choice of foods used in DBPCFCs is based on history, skin test RAST results, and/or foods suspected on the basis of elimination diets.

The diagnosis of food allergy is a clinical exercise that requires a careful history, selective skin tests or RASTs in cases of suspected IgE-mediated disorders, appropriate exclusion diets, and blinded provocation challenges. Currently, there is no evidence of diagnostic utility for the following assays: quantitation of food-specific IgG or IgG4 antibodies or food antigen-antibody complexes, evidence of lymphocyte activation (^{3}H uptake, interleukin-2 production, leukocyte inhibitory factor production, etc.), or sublingual or intracutaneous provocation. In gastrointestinal disorders where pre- and postchallenge biopsy studies are required for diagnosis (e.g., malabsorption syndromes, including celiac disease), the challenge does not require blinding.

Assessment of Allergenicity to Genetically Modified Foods and Novel Proteins

S. Lehrer recounted the efforts undertaken to assess the potential allergenicity of genetically modified foods. Recently, the U.S. National Academy of Sciences and other national science academies reviewed the issue of genetically modified foods. The report cited good reasons for the development of the science of agricultural biotechnology. The potential to alter the food supply suggests the possiblity of developing less-expensive and healthier foods that could play a role in the elimination of deficiency diseases and aid in feeding the growing world population. There is also the potential to reduce chemical pesticide use and increase the productivity of land, thus protecting habitat for other species (National Research Council 2000b). Another panel of the National Academy of Sciences looked specifically at plants genetically modified to include pesticidal properties. This panel concluded that there was no evidence that the technology is unsafe. At the same time, the committee conveyed concerns with ecologic risks of gene spread from the genetically modified crop to wild relatives and the development of pesticide-resistant superweeds. They also have cited a number of theoretical health concerns, including changes in nutritional composition or availability of nutrients in food, production of toxins, and potential of developing more allergenic foods or novel allergens in new foods (National Research Council 2000a).

Allergenicity risk assessment for genetically modified foods. During the last 5 years, a number of national groups, governmental agencies, and industry organizations, as well as international organizations such as the FAO of the United Nations and WHO, have become interested in issues of allergy and allergenicity assessment of these new food products. These organizations have supported a number of meetings and reports in which allergy risk assessment for genetically modified foods was addressed. The potential alteration of allergens in foods concerned changes in endogenous protein levels, expression of known allergens in different foods, and the expression of novel proteins that may be allergenic. The U.S. Food and Drug Administration (U.S. FDA) held one of the earliest meetings (in 1994) to address these issues (Metcalfe et al. 1996). Following this meeting, a series of meetings held by different organizations resulted in an evolving decision-tree process that continues to be altered according to new information about allergen structure and activity. The most recent version of such a decision tree came out of a WHO workshop in 2001 and is shown in Figure 1 (FAO/WHO 2001).

At this time, decision processes for allergenicity assessment make the initial choice of study on the basis of the source of the gene, that is, whether it is from a known allergen source or an unknown allergen source (Figure 1). If the gene is from a known allergen source, solid-phase immunoassays can be used to determine whether a known allergen is expressed in the new product. This information can be used to make a judgment about the potential for allergenicity of the product and whether it should be produced. Conversely, if the protein is from an unknown allergenic source or a source with little human exposure information, the ability to assess the potential for allergenicity is more problematic. On the basis of their physical and chemical properties, it is possible to identify novel proteins with little resemblance to known allergens and to judge that such proteins would have a lower likelihood of allergenicity. However, the predictive value of such an assessment is unknown. As mentioned in a previous paper in this mini-monograph (Metcalfe 2003), amino acid sequence comparison with known allergens, particularly known allergenic epitopes, has also been considered. This is in the original decision tree presented by Metcalfe et al. in 1996 (Metcalfe et al. 1996).

The practice of allergy risk assessment for genetically modified foods and novel proteins has generally worked well but should be improved as our knowledge of food allergy increases. Although stability in processing and enzymatic digestion is useful, assessments are not well standardized and validated. Amino

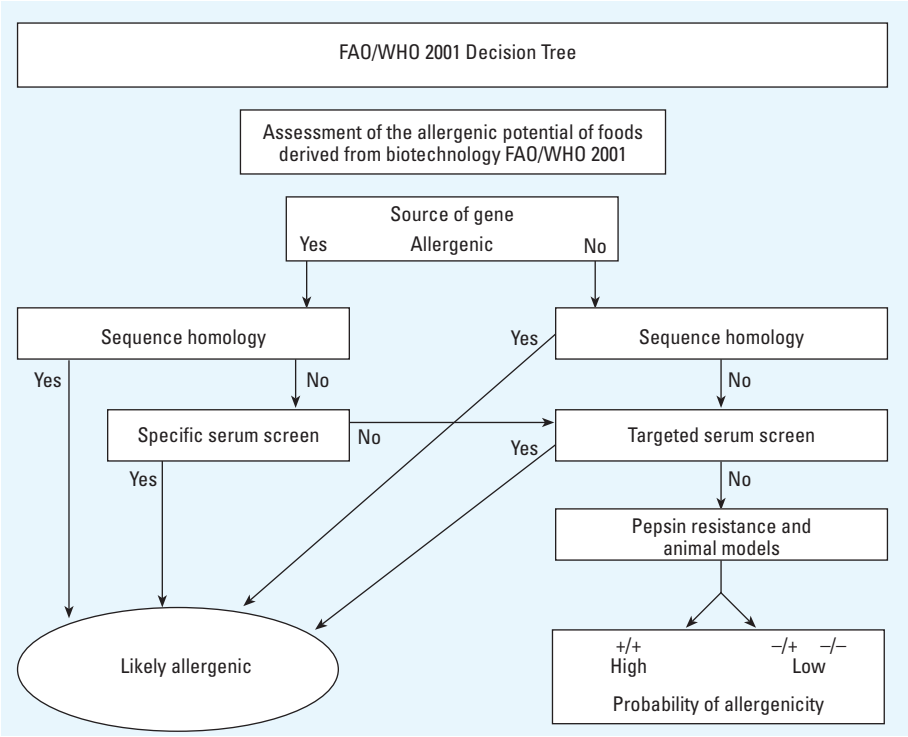

Figure 1. Decision-tree approach to determining the potential allergenicity of novel food products.

acid sequence similarity, as mentioned earlier, is being used more frequently, particularly as more allergens are sequenced. However, major questions concerning the comparison remain. Should it be the whole molecule or parts of the molecule? If parts of the molecule, should it be epitopes? What percent identity is important? All of these issues need to be resolved, and obtaining more information on allergen protein structure in particular will help make this process more precise.

A validated animal model would aid greatly in the assessment process. Such a model should respond to known food allergens in a manner similar to the human. One would expect that allergenic foods would have much more significant IgE reactivity than nonallergenic foods in such an animal model; allergenic proteins within the allergenic foods would have the same reactivity; and a pattern of reactivity to epitopes similar to that seen in man would be demonstrated in the animal.

The amount of food required for allergic sensitization or provocation in man is not known. This could provide important information, particularly if there is a level below that at which an allergen is not a risk. Several groups are addressing this process and should yield important information. Finally, more importance needs to be placed on the structure and sequence comparison, not only with allergens but also with nonallergens. If a novel protein with no background of allergenicity is very similar in structure and function to a nonallergen, should this not be an important lesson?

StarLink corn, produced by Aventis Corporation (Bridgewater Crossing, NJ) was approved by the U.S. EPA as a pesticide when produced for sale as an animal feed. In September 2000, it was reported and confirmed that StarLink had contaminated the human food supply. The basis for the approval process of StarLink only for animals was that it contained Cry9c, a protein from *Bacillus thuringiensis* (Bt), that appeared to be heat stable when compared with other Bt proteins and thus could not be excluded as an allergen (because allergenicity could not be ruled out), using an earlier version of the decision tree (Bucchini and Goldman 2002).

Because of the importance of this exposure, the U.S. FDA, the U.S. EPA, and the U.S. Department of Agriculture Food Safety and Inspection Service asked the National Food Processors Association (NFPA) to provide assistance in obtaining member company information related to potential adverse events that could conceivably be related to StarLink corn in processed foods. Eleven food companies submitted data to the NFPA on consumer contacts associated with processed foods containing yellow corn and possibly StarLink. Additional information estimating production units, total consumer contacts, allergy health contacts, and allergy-related product recalls was also provided. The U.S. Centers for Disease Control and Prevention (CDC) selected four time periods for review: 1998, when 10,000 acres of StarLink corn were planted; 1999, when 250,000 acres of StarLink corn were planted; and the year 2000 up to 17 September, when 350,000 acres of StarLink corn were planted. The fourth period was from 18 September through 11 November 2004, a 2-month period in which numerous product recalls ensued related to StarLink and thousands of media reports were issued.

The NFPA studies found no correlation between the amount of exposure to StarLink corn and the allergy/health contacts or allergy complaints to corn products. There appears to be a positive association between the number of allergy/health contacts for processed food containing yellow corn and the intense media coverage of StarLink-related product recalls.

Clinical and Laboratory Methods in the Assessment of the Allergenicity of Foods

R.G. Hamilton reviewed the methods for assessing food allergy. Any decision tree is only as good as the clinical and laboratory methods used in the assessment of the allergenicity of foods. The objective of this presentation was to discuss each component of the International Food Biotechnology Consortium (IFBC)/Allergy and Immunology Institute - International Life Sciences Institute (ILSI)/FAO/WHO Decision Tree (FAO/WHO 2001), with an emphasis on laboratory and clinical methods useful for investigating potential allergenicity of proteins in genetically modified foods.

Allergenicity has been defined as the ability to induce IgE antibodies that react with a protein (immunogenicity) or that cross-react with structurally similar epitopes. Allergenicity is determined by the antigenicity or foreignness of a molecule (size and stability), the extent of allergen exposure (allergen concentration), and the genetic predisposition of a host for inducing an allergic response (Hamilton 2001).

The IFBC/ILSI report estimates that approximately 160 foods and food-related substances are associated with allergic reactions (Metcalfe 1996). As of December 2001, 98 foods specificities have been U.S. FDA-cleared for IgE antibody measurement, and an additional 95 food specificities are in an allergen-specific reagent status for human IgE antibody testing with the Pharmacia CAP System.

The first level of the allergenicity assessment framework involves an examination of sequence homology to known allergens. What do we know about the predictive value of sequence homology? The primary structures of amino acid sequences deduced from cDNA are usually not complete because of posttranslational modifications (e.g., glycosylation of asparagines, serines, threonines; hydroxylation of prolines, lysines). Is the database of allergen amino acid sequences comprehensive? Aalberse and Stapel (2001) identified four structural families among 40 allergenic food proteins via 3D protein fold representations in 2D proximity plots. They concluded that allergens have no characteristic structural features other than they need to reach (and stimulate) immune cells and mast cells. Within this constraint, any antigen may be allergenic, particularly if it avoids activation of T-helper (Th)-2 suppressive mechanisms (CD8, Th1 cells).

Establishing serological assays for the detection of allergen-specific IgE antibody involves the use of a number of reagents (Hamilton 1997; Hamilton and Sobotka 2000). The first is a source of human IgE antibody. Pools of sera from well-characterized patients are needed, as IgE antibody levels are low (in the nanograms per milliliter range). The process of preparing serum pools dilutes out IgE antibodies with minor allergen specificities, so the process favors major allergen specificities. Such dilution is important, as biotechnology or other plant breeding methods theoretically can increase the expression of minor allergens, which would not be detected in such serum pools.

The second group of reagents needed for serological assays is a validated allergen source prepared by extracting the plant or food in a clearly defined manner with regard to extraction buffer, time, temperature, agitation, filtration, dialysis, and concentration (Hamilton and Sobotka 2000). This creates obvious challenges. Plant/food extraction conditions may vary widely, and such variability can increase the chance that cross-reactivity may occur. Cross-reactivity occurs when a protein other than the allergen source binds to the allergen-specific IgE. Cross-reactivity may or may not be of clinical significance. Pastorello et al. (2000) reported an experiment in which crude extracts of corn showed significant cross-reactivity with peach, which was not found with the purified corn protein. Such cross-reactivity can confuse the assessment of allergenicity.

Clinically, several U.S. FDA–cleared immunoassays are available for detecting allergen-specific IgE antibody in serum. These include the CAP System (Pharmacia), AlaSTAT (Diagnostic Products Corp., Los Angeles, CA), and Hy-TECH (Hycor, Garden Grove, CA). These clinical assays use a total serum IgE heterologous reference curve traceable to WHO IgE standard and quantitative IgE antibody results reported in kilo-international units per liter. There are also a number of research immunoassays that are less well characterized, including RAST, ELISA, and the immunoenzymetric assay (IEMA). Research IEMA assays that employ the native

and recombinant Cry proteins will be useful in detecting IgE antibodies specific for these proteins (Hamilton 1997, 2001; Hamilton and Sobotka 2000).

The clinical utility of assays for measuring food-specific IgE antibodies in serum is another important issue. A clinical diagnosis requires confirmation of clinical reactivity *in vivo*. Using DBPCFC, Sampson (2001) conducted a prospective study of children and adolescents and established 95% predictive decision points for egg-, milk-, peanut-, and fish-specific IgE antibody, as measured in the Pharmacia CAP System.

All of this is predicated on the notion that a food is a well-defined package of proteins and other constituents that can be easily characterized. However, we know that this is not the case. The experience with latex allergy in the United States illustrates the complexity of allergens in plant products. After universal precautions were instituted in 1986, there began an epidemic of allergic reactions and deaths associated with sensitization to a number of proteins in natural rubber products derived from latex of the *Hevea brasiliensis* tree (Hev b 1-Hev b 13). Use and exposure to latex in medical gloves was one major risk factor for latex allergen sensitization; sensitization was documented among 7% of health care workers and up to 50% of children with spina bifida. Cross-reactivity between latex proteins and certain food allergens was one of the factors that helped to identify latex-allergic individuals. Some of the common foods with defined cross-reactivity to latex are avocado, banana, chestnut, kiwi, raw potato, tomato, stone fruits (e.g., peach, cherry), hazelnut, melons, celery, carrot, apple, pear, papaya, and almond. Foods with less well-defined cross-reactivity to latex are peanut, peppers, citrus fruits, coconut, pineapple, mango, fig, passion fruit, ugli fruit, and grape (Salcedo et al. 2001).

From the latex experience, evidence has emerged for a family of proteinous plant panallergens, proteins that include profilin, patatin, plant stress proteins (WIN 1 and 2), and Bet v 1 and Bet v 2 (birch [*Betula verrucosa*] proteins). At present, thirteen allergenic *Hevea* proteins have been identified (cloned and sequenced) from latex, and these serve a variety of functions in the rubber tree. These proteins have quite different structures and molecular sizes. Breiteneder and Ebner developed a list of protein types among known food allergens (Breiteneder and Ebner 2000): *a*) pathogenesis-related proteins involved with defense against pests; *b*) seed-storage proteins; *c*) alpha amylase/protease inhibitors (wheat/barley/rye); *d*) Kunitz trypsin inhibitors (inhibit growth of larvae); *e*) thiol proteases (e.g., papain-papaya, ficin-fig, Gly m 1 soybean); *f*) profilins (12–15 kd actin cytoskeleton-binding protein, Ara h 5 peanut, Gly m 3 soybean, Hev b 8 latex; *g*) peroxidases (induced by pathogens in plant defense); and *h*) lectins (agglutinins that bind specific sugars on glycoproteins: 31 kDa peanut). It is evident that there will be no easy way to identify novel food allergens based on functional attributes of each protein. In addition, it is clear that some of the functional attributes of food allergens are those that may be the most desirable to plant breeders, for example, increasing the plant resistance to pests. Hanninen et al. observed in one experiment on turnips that plant stress can increase the concentrations of certain allergenic proteins (Hanninen et al. 1999). Can food processing increase the concentrations of allergenic proteins? One observation is that South American latex-allergic children with spina bifida routinely eat bananas without any apparent allergy problems. In contrast, latex-sensitized children (and adults) in North America often experience allergic reactions after eating bananas. In the United States, food distribution centers treat unripe bananas and other produce with ethylene to ripen; this is not commonly done in South America. Does treatment of food with ethylene induce banana proteins that cross-react with latex? Some research indicates that this can be the case for certain foods (Sanchez-Monge et al. 2000). Although we currently do not know the magnitude of this problem, it does illustrate another source of variability in the allergenicity of foods.

Can Postmarketing Surveillance Provide Useful Information about the Allergenicity of Genetically Modified Foods? The StarLink Corn Experience

C. Rubin outlined the situation with respect to postmarket surveillance for allergy to genetically modified foods. When a person manifesting signs or symptoms (e.g., hives, facial swelling, difficulty breathing) consistent with acute allergic reaction to a food product seeks medical care, the treatment is usually symptomatic, the episode is often isolated, and the potential allergen is seldom identified. The number of such medical visits is not tabulated. Even repeat visits for allergic reactions to well-known allergens such as peanuts or milk are not counted as part of any established surveillance system. Thus, in October 2000, when StarLink corn intended only for animal consumption was identified in products on grocery-store shelves, there was no way to easily determine if consumption of this genetically engineered protein was resulting in allergic reactions in humans (Bucchini and Goldman 2002).

In a coordinated effort to determine if StarLink corn was indeed responsible for adverse human health effects, the U.S. FDA and the CDC used existing postmarket surveillance resources to follow up people who self-identified as experiencing allergic reactions (CDC 2001). After media reports about StarLink, individuals in 18 states and territories called the U.S. FDA to report health effects potentially related to ingesting a corn product. Fifty-one people reported signs and symptoms that varied from gastrointestinal illness to anaphylactic shock. These reports were entered into the adverse events reporting system (AERS) that the U.S. FDA has used since 1997 to passively collect information about adverse human health effects related to the use of drugs marketed in the United States. Self- or physician-reports to AERS regarding food-related illness generate a standardized form that chronicles food consumption history, details the timing and manifestations of the adverse health event, and describes any medical treatment. The U.S. FDA aggregated all of the AERS reports received between 1 July and 30 November 2000 that mentioned consumption of a corn product. With all personal identifiers removed, these reports were sent to CDC for review to determine the likelihood that any of the reports were potentially related to StarLink.

CDC developed a case definition that included *a*) a suspected anaphylactic reaction (e.g., dizziness, weakness, or loss of consciousness) that occurred within 1 hr of product consumption; or *b*) any of the following dermatological or oropharyngeal symptoms (hives, rash, pruritus, oropharyngeal tingling or swelling) that occurred within 12 hr of product consumption; or *c*) any of the following gastrointestinal symptoms (vomiting, diarrhea, abdominal cramping) that occurred within 12 hr of product consumption and that involved only one individual among meal companions. It was also necessary that these symptoms were not explained by a preexisting medical condition.

Twenty-eight of the 51 reports were consistent with the case definition. The U.S. FDA then contacted each potential case person and requested permission for CDC to follow up the adverse health event. Each potential case was administered a detailed questionnaire, gave consent to obtain medical records, and was also asked to contribute a serum sample that would be stored until a test could be developed to measure IgE antibodies to Cry9c protein. Although all 28 people appeared to have clinically experienced an immediate hypersensitivity to an allergen, our postmarketing surveillance could not demonstrate that the Cry9c protein was actually in the product consumed prior to the adverse health event. A serological test for antibodies

specific to acute hypersensitivity was considered to be the safest way to evaluate whether any of these people were indeed sensitive to the genetically modified protein in StarLink. The U.S. FDA developed an ELISA test that found no IgE antibody reactivity to Cry9c in any of the serum samples. This test was limited because the Cry9c used in the ELISA was a recombinant protein produced in bacteria rather than the protein expressed in plants. Concern that this difference may alter the protein's allergenicity has encouraged researchers to work on developing additional tests to further demonstrate the lack of reactivity to the Cry9c protein. This work is in progress, and the results will be published in the near future.

The StarLink experience demonstrates many of the limitations in using postmarket surveillance for adverse reactions to food as a method for assessing allergenicity to a protein that has been newly introduced into the food supply. Intensive epidemiologic investigation and laboratory test development by federal investigators was not sufficient to determine whether individual allergic reactions were associated with the inadvertent release of a genetically modified protein into the human food supply. It is also unlikely that postmarketing physicians or hospital-based surveillance would have been able to detect any increase in allergic reactions during the time that StarLink corn was available to consumers. The symptoms described in the case definition used in this investigation are generic and could have been attributed to a variety of etiologies. The StarLink example demonstrates many of the problems with any surveillance system that tries to capture rare and somewhat generic health events such as food allergy.

Is Susceptibility to Food Allergy in Workers Sensitized by Inhalant Exposure to *B. thuringiensis* kurstaki a Relevant Model for Sensitivity to Genetically Modified Foods?

L. Bernstein reviewed the occupational allergy experience with inhalation of Bt proteins. Novel proteins in genetically modified food may be present in the dust in workplaces where such foods are processed or handled. Novel proteins in genetically modified food may be present in the dust in workplaces where such foods are processed or handled. Thus, occupational populations may be exposed via inhalation. The possibilities of prior exposure and allergic sensitization to proteins that are potentially cross-reactive with novel proteins contained in genetically modified foods should be considered relevant risk factors prior to general introduction of such foods into the human food chain. By serendipity, this hypothetical circumstance actually occurred before StarLink corn was inadvertently discovered in food products generally available to human consumers.

In 1999, researchers discovered that migrant health workers developed positive skin tests and elevated specific IgE and IgG antibody levels to *B. thuringiensis* kurstaki (Btk) spore extracts containing Cry1Aa and Cry1Ab delta endotoxin proteins after respiratory exposure to Btk crop spraying (Bernstein et al. 1999). A number of positive skin tests, as well as increased levels of specific IgE and IgG antibodies, were present in more highly exposed groups than in medium- to low-exposure groups. Preexisting atopy was also a risk factor for workers with positive skin and serologic tests in the medium- and low-exposure groups. Although consumers frequently use Btk spray products for gardening purposes, similar risk assessment studies in this population have not been investigated.

Because of the documented success of Btk delta endotoxin insecticides in the control of corn borer larvae, investigators postulated that direct incorporation of genes encoding these proteins into corn and maize seeds would further enhance productivity of these commodities. *Cry1Aa* and *Cry1Ab* genes were among the first *Btk* genes to be used for this purpose. Later, the *Cry9C* gene, uniquely modified to enable persistence of the encoded protein in the larval gut, was used in StarLink transgenic corn seed. Although immunologic cross-reactivity between Cry1A and Cry9C proteins has not yet been investigated, it is of particular interest that these proteins share proteomic homology in 75% of their conserved amino acid residues as well as identity of the chief domains that determine tertiary structure of these proteins.

For the above reasons and in view of prior experience with respiratory occupational exposure to Btk in migrant workers, researchers extensively reviewed the literature concerning the occurrence of subsequent food allergy in workers previously sensitized after respiratory exposure to a variety of food proteins (Cartier et al. 1984; Leser et al. 2001; Lybarger et al. 1982; Smith et al. 1987). Unfortunately, there were a limited number of cross-sectional studies of occupational asthma (OA) induced by inhaled food proteins where the question of subsequent food allergy was addressed. However, in plants processing foods such as eggs, snow crabs, and condiments, 18 (35%) of 51 workers with confirmed OA developed food allergy symptoms at varying intervals after the onset of OA (Table 3).

A future approach to the potential allergenicity of genetically modified foods during postmarketing surveillance should include human susceptibility and risk factors in atopic subset populations; prior occupational exposure and sensitization to related proteins; and prior consumer exposure and sensitization to related proteins.

Occupational exposure to novel proteins and potential sensitization is an issue that has had little study, yet could be of public health significance. Approaches are available for further study, and exposed cohorts are available.

Conclusions

The introduction of genetically modified foods into the marketplace has brought to light the challenges inherent in identification of food allergens and individuals who are sensitive to those allergens. Although theoretically any plant-breeding technique can modify (increase or decrease) the allergenicity of foods, biotechnology has a greater potential to introduce novel proteins into the food supply, and thus has been subjected to closer scrutiny by regulators. Expert bodies have developed a decision tree for assessment of food allergy risks from such foods. All aspects of current food allergy assessments, both clinical and laboratory tools, have technical challenges that must be addressed if such tools are brought into a regulatory context. However, our challenge scientifically is how to assess novel proteins that have little or no exposure in the general population and thus no readily available tools for the prediction of exposures. The experiences with StarLink corn and in occupational cohorts exposed to grain dusts suggest that the development of methods to be used for postmarket consumer and occupational health surveillance may be useful. Thus, the current FAO/WHO decision tree for assessment of food allergy risks may require revision to include evaluation of appropriate diagnostic tests in these susceptible population groups, and to address technical challenges in assessing proteins newly introduced into the diet. Later papers in this monograph suggest research strategies for development of tools that may be useful for the prediction and/or postmarket surveillance of allergy to novel proteins in foods.

Table 3. Prevalence of food allergy to foods that induce occupational asthma by inhalation.

Allergen	Suspected OA (*n*)	Confirmed OA (*n*)	Food allergy (*n*)	Reference
Egg	29	8	4	Smith et al. 1987; Leser et al. 2001
Snow crab	103	37	12	Cartier et al. 1984
Garlic	3	3	1	Lybarger et al. 1982
Condiments	3	3	1	Bernstein et al. Unpublished observations
Total	138	51	18 (35%)	

References

Aalberse RC, Stapel SO. 2001. Structure of food allergens in relation to allergenicity. Pediatr Allergy Immunol 12:10–14.

Bernstein IL, Bernstein JA, Miller M, Tierzieva S, Bernstein DI, Lummus Z, et al. 1999. Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. Environ Health Perspect 107:575–582.

Bock SA. 1987. Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. Pediatrics 79:683–688.

———. 1992. Respiratory reactions induced by food challenges in children with pulmonary disease. Pediatr Allergy Immunol 3:188–194.

Breiteneder H, Ebner C. 2000. Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immunol 106:27–36.

Bucchini L, Goldman LR. 2002. Starlink corn: a risk analysis. Environ Health Perspect 110:5–13.

Burks AW, Mallory SB, Williams LW, Shirrell MA. 1988. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. J Pediatr 113:447–451.

Cartier A, Malo JL, Forest F, Lafrance M, Pineau L, St-Aubin JJ, et al. 1984. Occupational asthma in snow crab-processing workers. J Allergy Clin Immunol 74:261–269.

CDC. 2001. Investigation of Human Health Effects Associated with Potential Exposure to Genetically Modified Corn: A Report to the U.S. Food and Drug Administration from the Centers for Disease Control and Prevention. Atlanta, GA:Centers for Disease Control and Prevention.

Champion RH, Roberts SO, Carpenter RG, Roger JH. 1969. Urticaria and angio-oedema. A review of 554 patients. Br J Dermatol 81:588–597.

FAO/WHO. 2001. Evaluation of Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO Expert Consultation of Allergenicity of Foods Derived from Biotechnology. Available: http://www.fao.org/es/esn/gm/allergygm.pdf [accessed 11 September 2002].

Hall RP. 1987. The pathogenesis of dermatitis herpetiformis: recent advances. J Am Acad Dermatol 16:1129–1144.

Hamilton RG. 1997. Immunological tests for the diagnosis and management of human allergic disease: total and allergen-specific IgE and allergen-specific IgG. In: Manual of Clinical Laboratory Immunology (Rose HR, de Macario EC, Fahey JL, Friedman H, Penn GM, eds). Washington, DC:American Society for Microbiology, 881.

———. 2001. Assessment of allergic diseases. In: Clinical Immunology (Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Kotzin B, eds). 2nd ed. London:Harcourt Health Sciences, 124.1–124.14.

Hamilton RG, Sobotka AK. 2000. *In vitro* diagnostic tests of IgE-mediated reactions. In: Diagnostic Testing of Allergic Diseases (Kemp SF, Lockey RF, eds). New York:Marcel Dekker, 89–110.

Hanninen AR, Mikkola JH, Kalkkinen N, Turjanmaa K, Ylitalo L, Reunala T, et al. 1999. Increased allergen production in turnip (*Brassica rapa*) by treatments activating defense mechanisms. J Allergy Clin Immunol 104:194–201.

Hide DW, Guyer BM. 1983. Cows milk intolerance in Isle of Wight infants. Br J Clin Pract 37:285–287.

Horan R, Sheffer A. 1991. Food-dependent exercise-induced anaphylaxis. Immunol Allergy Clin North Am 11:757–766.

Host A, Husby S, Osterballe O. 1988. A prospective study of cow's milk allergy in exclusively breast-fed infants. Incidence, pathogenetic role of early inadvertent exposure to cow's milk formula, and characterization of bovine milk protein in human milk. Acta Paediatr Scand 77:663–670.

Iacono G, Carroccio A, Cavataio F, Montalto G, Kazmierska I, Lorello D, et al. 1996. Gastroesophageal reflux and cow's milk allergy in infants: a prospective study. J Allergy Clin Immunol 97:822–827.

James JM, Bernhisel-Broadbent J, Sampson HA. 1994. Respiratory reactions provoked by double-blind food challenges in children. Am J Respir Crit Care Med 149:59–64.

James JM, Eigenmann PA, Eggleston PA, Sampson HA. 1996. Airway reactivity changes in asthmatic patients undergoing blinded food challenges. Am J Respir Crit Care Med 153:597–603.

Jansen JJ, Kardinaal AF, Huijbers G, Vlieg-Boerstra BJ, Martens BP, Ockhuizen T. 1994. Prevalence of food allergy and intolerance in the adult Dutch population. J Allergy Clin Immunol 93:446–456.

Kelly KJ, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. 1995. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. Gastroenterology 109:1503–1512.

Kemp SF, Lockey RF, Wolf BL, Lieberman P. 1995. Anaphylaxis. A review of 266 cases. Arch Intern Med 155:1749–1754.

Kuitunen P, Visakorpi JK, Savilahti E, Pelkonen P. 1975. Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. Arch Dis Child 50:351–356.

Lee CM, Changchien CS, Chen PC, Lin DY, Sheen IS, Wang CS, et al. 1993. Eosinophilic gastroenteritis: 10 years experience. Am J Gastroenterol 88:70–74.

Leser C, Hartmann AL, Praml G, Wuthrich B. 2001. The "egg-egg" syndrome: occupational respiratory allergy to airborne egg proteins with consecutive ingestive egg allergy in the bakery and confectionery industry. J Invest Allergol Clin Immunol 11:89–93.

Lybarger JA, Gallagher JS, Pulver DW, Litwin A, Brooks S, Bernstein IL. 1982. Occupational asthma induced by inhalation and ingestion of garlic. J Allergy Clin Immunol 69:448–454.

Machida HM, Catto Smith AG, Gall DG, Trevenen C, Scott RB. 1994. Allergic colitis in infancy: clinical and pathologic aspects. J Pediatr Gastroenterol Nutr 19:22–26.

Metcalfe DD. 2003. Introduction: what are the issues in addressing the allergenic potential of genetically modified foods? Environ Health Perspect 111:1110–1113.

Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, Fuchs RL. 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Crit Rev Food Sci Nutr 36:S165–S186.

Min KU. 1991. Eosinophilic gastroenteritis. Immunol Allergy Clin North Am 11:799–813.

Nagata S, Yamashiro Y, Ohtsuka Y, Shioya T, Oguchi S, Shimizu T, et al. 1995. Quantitative analysis and immunohistochemical studies on small intestinal mucosa of food-sensitive enteropathy. J Pediatr Gastroenterol Nutr 20:44–48.

National Research Council. 2000a. Genetically Modified Pest-Protected Plants: Science and Regulation. Washington, DC:National Academy Press.

———. 2000b. Transgenic Plants and Worldwide Agriculture. Washington, DC:National Academy Press.

Novembre E, de Martino M, Vierucci A. 1988. Foods and respiratory allergy. J Allergy Clin Immunol 81:1059–1065.

Odze RD, Wershil BK, Leichtner AM, Antonioli DA. 1995. Allergic colitis in infants. J Pediatr 126:163-170.

Oehling A, Baena Cagnani CE. 1980. Food allergy and child asthma. Allergol Immunopathol (Madr) 8:7–14.

Ortolani C, Ispano M, Pastorello E, Bigi A, Ansaloni R. 1988. The oral allergy syndrome. Ann Allergy 61:47–52.

Pastorello EA, Farioli L, Pravettoni V, Ispano M, Scibola E, Trambaioli C, et al. 2000. The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein. J Allergy Clin Immunol 106:744–751.

Pastorello EA, Ortolani C, Farioli L, Pravettoni V, Ispano M, Borga A, et al. 1994. Allergenic cross-reactivity among peach, apricot, plum, and cherry in patients with oral allergy syndrome: an *in vivo* and *in vitro* study. J Allergy Clin Immunol 94:699–707.

Powell GK. 1978. Milk- and soy-induced enterocolitis of infancy. Clinical features and standardization of challenge. J Pediatr 93:553–560.

Romano A, Di Fonso M, Giuffreda F, Quaratino D, Papa G, Palmieri V, et al. 1995. Diagnostic work-up for food-dependent, exercise-induced anaphylaxis. Allergy 50:817–824.

Salcedo G, Diaz-Perales A, Sanchez-Monge R. 2001. The role of plant panallergens in sensitization to natural rubber latex. Curr Opin Allergy Clin Immunol 1:177–183.

Sampson H. 1993. Adverse reactions to foods. In: Allergy: Principles and Practice (Middleton E, Reed CE, Elliset EF, eds). 4th ed. St. Louis:C.V. Mosby Co., 1661–1686.

Sampson HA. 1989. Infantile colic and food allergy: fact or fiction? J Pediatr 115:583–584.

———. 1992. The immunopathogenic role of food hypersensitivity in atopic dermatitis. Acta Derm Venereol Suppl 176:34–37.

———. 2001. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 107:891–896.

Sampson HA, McCaskill CC. 1985. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. J Pediatr 107:669–675.

Sampson HA, Mendelson L, Rosen JP. 1992. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med 327:380–384.

Sampson HA, Scanlon SM. 1989. Natural history of food hypersensitivity in children with atopic dermatitis. J Pediatr 115:23–27.

Sanchez-Monge R, Blanco C, Perales AD, Collada C, Carrillo T, Aragoncillo C, et al. 2000. Class I chitinases, the panallergens responsible for the latex-fruit syndrome, are induced by ethylene treatment and inactivated by heating. J Allergy Clin Immunol 106:190–195.

Schrander JJ, van den Bogart JP, Forget PP, Schrander-Stumpel CT, Kuijten RH, Kester AD. 1993. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. Eur J Pediatr 152:640–644.

Smith AB, Bernstein DI, Aw TC, Gallagher JS, London M, Kopp S, et al. 1987. Occupational asthma from inhaled egg protein. Am J Ind Med 12:205–218.

Snyder JD, Rosenblum N, Wershil B, Goldman H, Winter HS. 1987. Pyloric stenosis and eosinophilic gastroenteritis in infants. J Pediatr Gastroenterol Nutr 6:543–547.

Volonakis M, Katsarou-Katsari A, Stratigos J. 1992. Etiologic factors in childhood chronic urticaria. Ann Allergy 69:61–65.

Waldman T, Wochner R, Laster R, Gordon RS. 1967. Allergic gastroenteropathy: a cause of excessive gastrointestinal protein loss. N Engl J Med 276:761–769.

Yocum MW, Khan DA. 1994. Assessment of patients who have experienced anaphylaxis: a 3-year survey. Mayo Clin Proc 69:16–23.

Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. 1994. A population study of food intolerance. Lancet 343:1127–1130.

Yunginger JW, Sweeney KG, Sturner WQ, Giannandrea LA, Teigland JD, Bray M, et al. 1988. Fatal food-induced anaphylaxis. JAMA 260:1450–1452.