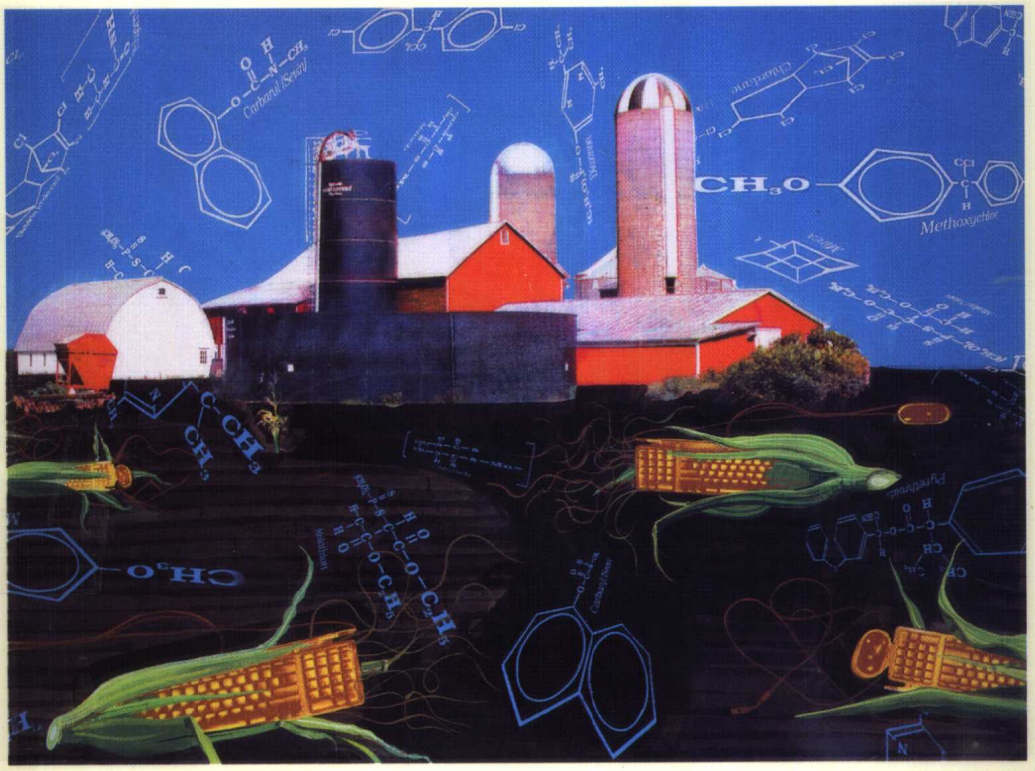
GENETICALLY ENGINEERED FOODS

ASSESSING POTENTIAL ALLERGENICITY



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Edited by Tong-Jen Fu and Steven M. Gendel

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Preface

Advances in biotechnology have resulted in an increasing number of genetically engineered foods that contain proteins from sources that have not been part of the food supply. The allergenic potential of the new gene products needs to be assessed to ensure the safety of these foods. The strategy adopted by the biotechnology industry to assess the allergenic potential of genetically engineered foods, as a part of the overall food safety evaluation process, is to compare the properties of novel proteins with those of known allergens, and evaluate the allergic potential based on whether the transgenic proteins share similar properties with those of known allergens. In 1996, on the basis of then-available data, the International Food Biotechnology Council (IFBC) and the International Life Sciences Institute (ILSI), Allergy and Immunology Institute jointly developed a decision-tree approach for the assessment of the allergenic potential of genetically engineered foods. The 1996 IFBC/ILSI decision tree, although widely adopted by the agricultural biotechnology industry, has received some criticism with respect to certain criteria (or the lack of them) set forth.

In recent years, increasing knowledge regarding food allergies, the properties of food allergens, and new approaches for allergenicity testing of foods have become available in the literature. A need exists to determine whether the results of this recent research can be incorporated into the current decision-tree approach, whether there is enough information to support the use of additional methodologies for assessing the allergenic potential of transgenic proteins, and what other research is needed.

To address this need, a symposium entitled "Assessment of the Potential Allergenicity of Genetically Engineered Foods" was organized and held on December 5-6, 2000 at the National Center for Food Safety and Technology in Summit, Illinois. In this symposium, international experts from academia, government, and industry summarized the current understanding of food allergy and food allergens, commented on the current decision-tree approach for assessing the potential allergenicity of proteins, and discussed alternative testing methods for assessing the potential allergenicity of transgenic proteins. Meeting participants also discussed whether and how the newly acquired information might be incorporated into the current assessment strategy and made recommendations on future research needs.

This volume, composed mostly of papers presented at the symposium, provides a summary of the science and the issues important to the development of strategies for assessing the potential allergenicity of foods derived from biotechnology. It is hoped that it will be a useful resource to stimulate interest in continuing research in this area. This volume should be of interest to scientists involved in food allergy research and to individuals in the industry and

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regulatory agencies who are involved in the safety evaluation of genetically engineered foods.

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—TONG-JEN FU
—STEVEN M. GENDEL

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This volume is the result of a conference entitled **Assessment of the Potential Allergenicity of Genetically Engineered Foods,** sponsored by the National Center for Food Safety and Technology, and held on December 5–6, 2000 in Summit, Illinois.

CONTENTS

Part III. Other Alternatives for Testing Allergenicity

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Current Understanding of Food Allergy

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ABSTRACT: **IgE-mediated hypersensitivity reactions account for the majority of well-documented food allergy reactions, but non-IgE-mediated immune mechanisms do cause some hypersensitivity disorders. A variety of gastrointestinal, cutaneous, respiratory, and generalized symptoms and syndromes have been associated with IgE-mediated food allergy. The diagnostic approach to adverse food reactions begins with a careful medical history and physical examination. Laboratory studies may then be used appropriately in the evaluation. Once the diagnosis of food allergy is established, the only proven therapy is the strict elimination of the food from the patient's diet. Studies in both children and adults indicate that symptomatic reactivity to food allergens is often lost over time, except possibly reactions to peanuts, tree nuts, and seafood.**

KEYWORDS: **food hypersensitivity; food intolerance; IgE-mediated gastrointestinal allergy; radioallergosorbent tests (RASTs); adverse food reaction; food allergy**

Critical to any discussion of food allergy and food intolerance is a basic understanding of the classification of adverse food reactions.[1] The precise use of these terms has allowed better communication in the scientific literature regarding various reactions to food components. An *adverse food reaction* is a general term that can be applied to a clinically abnormal response to an ingested food or food additive. Adverse food reactions may be secondary to *food hypersensitivity (allergy)* or *food intolerance.*

Food hypersensitivity (allergy) is an immunologic reaction resulting from the ingestion of a food or food additive. This reaction occurs only in some patients, may occur after only a small amount of the substance is ingested, and

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is unrelated to any physiologic effect of the food or food additive. To most physicians, the term is synonymous with reactions that involve the immunoglobulin E (IgE) mechanism, of which anaphylaxis is the classic example.

Food intolerance is a general term describing an abnormal physiologic response to an ingested food or food additive. This reaction has not been proven to be immunologic in nature and may be caused by many factors, including toxic contaminants (e.g., histamine in scombroid fish poisoning and toxins secreted by salmonella, shigella, and campylobacter), pharmacologic properties of the food (e.g., caffeine in coffee and tyramine in aged cheeses), characteristics of the host such as metabolic disorders (e.g., lactase deficiency), and idiosyncratic responses.

The term food intolerance has often been overused and, like the term food allergy, has been applied incorrectly to all adverse reactions to foods. IgE-mediated (Type I) hypersensitivity accounts for the majority of well-characterized food allergic reactions, although non-IgE-mediated immune mechanisms are believed to be responsible for a variety of hypersensitivity disorders. For this discussion we will examine adverse food reactions that are IgE mediated, non-IgE mediated, and those entities that have characteristics of both.

CLINICAL MANIFESTATIONS OF FOOD HYPERSENSITIVITY

IgE-Mediated Hypersensitivity

Gastrointestinal Food Hypersensitivity Reactions

The signs and symptoms of food-induced IgE-mediated gastrointestinal allergy in humans may be secondary to a variety of syndromes, including the oral allergy syndrome, immediate gastrointestinal hypersensitivity, and a small subgroup of allergic eosinophilic gastroenteritis.

The *oral allergy syndrome* (TABLE 1) is considered a form of contact urticaria that is confined almost exclusively to the oropharynx and rarely involves other target organs. The symptoms include rapid onset of pruritus and angioedema of the lips, tongue, palate, and throat. The symptoms generally resolve quite rapidly. This syndrome is most commonly associated with the ingestion of fresh fruits and vegetables. Interestingly, patients with allergic rhinitis secondary to certain airborne pollens (especially ragweed and birch pollens) are frequently afflicted with this syndrome. Patients with ragweed allergy may experience these symptoms following contact with certain melons (watermelons, cantaloupe, honeydew, etc.) and bananas. Those patients with birch sensitivity often have symptoms following the ingestion of raw potatoes, carrots, celery, apples, and hazelnuts. The diagnosis of this syndrome

TABLE 1. Oral allergy syndrome

Oral manifestations	
	Burning
	Swelling
	Itching
	Erythema
	Immediate onset of symptoms
Age of onset	
	Beyond infancy
	Typically less than 5 years
Proteins implicated	
	Heat-labile fresh fruit and vegetable allergens
	Pollen and latex cross-reactivity
Pathology	
	Immunoglobulin E antibodies
Treatment	
	Avoidance
	Cooking
Natural history	
	Unknown

SOURCE: From Sampson and Anderson,[18] p. S88. Reproduced by permission.

is made after a suggestive history and positive skin-prick tests with the implicated fresh fruits or vegetables.[2] The difficulty in diagnosing this syndrome is that the commercially available allergen extracts for fresh fruits and vegetables often do not have the reliability of the other food extracts. It may be necessary to use the"prick-by-prick" method, where the device used for introducing the allergen into the skin may have to initially be "pricked" into the food.

Immediate gastrointestinal hypersensitivity (TABLE 2) is a form of IgE-mediated gastrointestinal hypersensitivity that may accompany allergic manifestations in other target organs.[3] The symptoms vary but may include nausea, abdominal pain, abdominal cramping, vomiting, and/or diarrhea. In studies of children with atopic dermatitis and food allergy, the frequent ingestion of a food allergen appears to induce partial desensitization of gastrointestinal mast cells, resulting in less pronounced symptoms (as suggested in the rodent model).

The diagnosis of this disorder is made by a suggestive clinical history, positive skin-prick tests, complete elimination of the suspected food allergen for up to two weeks with resolution of symptoms, and oral food challenges. After avoidance of a particular food for 10–14 days, it is not unusual for symptoms of vomiting to occur during a challenge even though the patient was previously ingesting the food without vomiting each time they ate it.

TABLE 2. Immediate gastrointestinal hypersensitivity

Manifestations	
	Nausea, abdominal pain and vomiting within 1 to 2 hours
	Diarrhea within 2 to 6 hours
	Frequently associated with atopic disease
	Food-specific IgE antibodies
	Radiographic: gastric hypotonia and pylorospasm
Age of onset	
	Infancy, childhood
Proteins implicated	
	Milk, egg, peanut, soy, cereal, fish
Pathology	
	Immunoglobulin E–mediated
Treatment	
	Protein elimination
Natural history	
	80% of cases resolve after protein elimination diet (except in the case of peanut and fish allergy)

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Mixed IgE-Mediated and Non-IgE-Mediated

Allergic eosinophilic gastroenterocolitis (TABLES 3 and 4) is a disorder characterized by infiltration of the gastric and/or intestinal walls with eosinophils, absence of vasculitis, and, frequently, peripheral eosinophils.[4] Patients presenting with this syndrome frequently have postprandial nausea and vomiting, abdominal pain, diarrhea, occasionally steatorrhea, and failure to thrive in young infants or weight loss in adults. There appears to be a subset of patients with allergic eosinophilic gastroenteritis who have symptoms secondary to food. These patients generally have the mucosal form of this disease with IgE-staining cells in jejunal tissue, elevated IgE in duodenal fluids, atopic disease, elevated serum IgE concentrations, positive skin-prick tests to a variety of foods and inhalants, peripheral blood eosinophil iron deficiency anemia, and hypoalbuminemia.

The diagnosis of this entity is based on an appropriate history and a gastrointestinal biopsy demonstrating a characteristic eosinophilic infiltration. Multiple sites (up to 8) may need to be biopsied to effectively exclude eosinophilic gastroenteritis because the eosinophilic infiltrates may be quite patchy.[5] Patients with the mucosal form of the disease may have atopic symptoms, including food allergy, elevated serum IgE concentrations, positive skin tests or RASTs, and peripheral eosinophillia. Other laboratory studies consistent with this disease include Charcot-Leyden crystals in the stool, anemia, hypoalbuminemia, and abnormal D-xylose tests. An elimination diet of up to

TABLE 3. Allergic eosinophilic gastritis

Manifestations
Vomiting
Abdominal pain
Anorexia
Early satiety
Hematemesis
Failure to thrive
Gastric outlet obstruction
Gastric bleeding
50% of cases atopic
Elevated immunoglobulin E
50% of cases with peripheral eosinophilia
Radiographic: antral obstruction,thickened folds
Gastroesophageal reflux
Failure to respond to H-2 blockers
Responds to protein elimination
Age at onset
Neonate to adolescent
Proteins implicated
Cow's milk, egg, corn, cod, soy
Often single antigen
Less than 50% skin test specificity
Pathology
Marked eosinophilic infiltration of gastic mucosa, submucosa, especially in the gastric antrum
Treatment
Protein elimination
Excellent response to hydrolyzed protein formula in patients less than 2 years of age
Excellent response to L-amino acid formula
Excellent response to low-dose, often long-term, steroids
Natural history
Guarded outcome in older patients

SOURCE: From Sampson and Anderson,[18] p. S90. Reproduced with permission.

12 weeks may be necessary before complete resolution of symptoms and normalization of intestinal histology.

Non-IgE-Mediated Food Hypersensitivity

Dietary protein enterocolitis (also known as protein intolerance) (TABLE 5) is a disorder that presents most commonly in young infants between 1 week and 3 months of age. The typical symptoms are isolated to the gastrointestinal tract and typically consist of recurrent vomiting and/or diarrhea.[6] The symp-

TABLE 4. Allergic eosinophilic gastroenterocolitis

Manifestations
Abdominal pain
Anorexia
Early satiety
Failure to thrive
Gastric outlet obstruction
Gastric or colonic bleeding
±70% of cases atopic
Elevated immunoglobulin E
±Food-specific immunoglobulin E
50% of cases with peripheral eosinophilia
Radiographic: antral obstruction, Menetrier's disease, gastroesophageal reflux, bowl wall edema, vomiting, diarrhea, protein-losing enteropathy, decreased albumin
Age at onset
Neonate to adolescent
Proteins implicated
Cow's milk, egg, fish, soy, cereals
Less than 50% skin test specificity
Pathology
Marked eosinophilic infiltration of mucosaand submucosa; gastic antrum, esophagus, and duodenum; and colon
Treatment
50% of patients respond to dietary elimination of documented allergen
Excellent response to hydrolyzed protein formula in patients less than 2 years of age
Excellent response to L-amino acid formula
Responsive to steroids
Natural history
Disorder typically prolonged

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toms can be severe enough to cause dehydration. Cow's milk and soy protein (particularly in infant formulas) are most often responsible for this syndrome, although egg sensitivity has been reported in older patients. The children will often have stools that contain occult blood, polymorphonuclear neutrophils, and eosinophils and are frequently positive for reducing substances (indicating malabsorbed sugars). Skin-prick tests for the putative food protein are characteristically negative. Jejunal biopsies classically reveal flattened villi, edema, and increased numbers of lymphocytes, eosinophils, and mast cells. A food challenge with the responsible protein generally results in vomiting and/or diarrhea within minutes to several hours, occasionally leading to shock. It is not uncommon to find children who are sensitive to both cow's milk and soy protein. This disorder also tends to be lost by 18–24 months of age.

TABLE 5. Dietary protein enterocolitis

Manifestations
Diarrhea with bleeding
Anemia
Emesis
Abdominal distension
Failure to thrive
Hypotension
Fecal leukocytes
Normal immunoglobulin E
Food challenge: vomiting in 3 to 4 hours; diarrhea in 5 to 8 hours
Age at onset
1 day to 1 year
Implicated proteins
Cow's milk, soy, rice, poultry, fish
Pathology
Patchy villous injury and colitis
Treatment
80% or more of cases respond to hydrolyzed casein formula and symptoms clear in 3 to 10 days
Up to 20% of cases require L-amino acid formula or temporary intravenous therapy
Natural history
Generally: with treatment 50% of cases resolve by 18 months; 90% of cases resolve by 36 months
Cow's milk: with treatment 50% of cases resolve by 18 months; 90% of cases resolve by 36 months
Soy: illness is often more persistent

SOURCE: From Sampson and Anderson,[18] p. S91. Reproduced with permission.

Elimination of the offending allergen generally will result in improvement or resolution of the symptoms within 72 hours, although secondary disaccharidase deficiency may persist longer.[6] Oral food challenges, which should be done in a medical setting because they can induce severe vomiting, diarrhea, dehydration, or hypotension, consist of administering 0.6 gm/kg body weight of the suspected food allergen.[6]

Dietary protein proctitis (TABLE 6) generally presents in the first few months of life and is often secondary to cow's milk or soy protein hypersensitivity.[7] Infants with this disorder often do not appear ill, have normally formed stools, and generally are discovered because of the presence of blood (gross or occult) in their stools. Gastrointestinal lesions are confined to the small bowel and consist of mucosal edema with eosinophils in the epithelium and lumina propria. If lesions are severe and exhibit crypt destruction, PMNs are also prominent.[8] It is thought, in the absence of a lot of well-controlled

TABLE 6. Dietary protein proctitis

Manifestations
Blood-streaked, soft-to-loose stools
Fecal leukocytes
Mild peripheral eosinophilia
Mild hypoalbuminemia
Low risk of anemia
Food challenge: symptoms in 6 to 72 hours
Age at onset
1 day to 6 months
Most cases manifest at 2 to 8 weeks
Proteins implicated
60% of reported infants exclusively breast fed
Cow's milk, egg, soy
Pathology
Endoscopic: focal to diffuse colitis, linear erosions
Microscopic: eosinophilic colitis more than 20 eosinophils/40 per high-power field; 20% of cases with nodular lymphoid hyperplasias
Treatment
Protein elimination
Symptoms generally clear in 72 hours in patients given extensively hydrolzed formula.
Resume or continue breastfeeding on maternal antigen-restricted diet
Natural history
Symptoms usually clear by one year

SOURCE: From Sampson and Anderson,[18] p. S91. Reproduced with permission.

studies, that cow's milk and soy protein–induced colitis resolves by 6 months to 2 years of allergen avoidance.

Elimination of the offending food allergen leads to resolution of hematochezia within 72 hours, but the mucosa lesions may take up to one month to disappear and range from patchy mucosal injection to severe friability with small aphthoid ulcerations and bleeding.

DIAGNOSING ADVERSE FOOD REACTIONS

As with all medical disorders, the diagnostic approach to the patient with a suspected adverse food reaction begins with the medical history and physical examination. Various laboratory studies, based on the information derived from these initial steps, may be helpful.

The true value of the medical history is largely dependent on the patient's recollection of symptoms and the examiner's ability to differentiate disorders provoked by food hypersensitivity and other causes. The history may be di-

rectly useful in diagnosing food allergy in acute events (e.g., systemic anaphylaxis following the ingestion of fish). In many series, however, less than 50% of reported food allergic reactions could be substantiated by DB-PCFC.[9,10] Several pieces of information are important to establish that a food allergic reaction has occurred: (1) the food suspected to have provoked the reaction, (2) the quantity of the food ingested, (3) the length of time between ingestion and development of symptoms, (4) a description of the symptoms provoked, (5) whether similar symptoms developed on other occasions when the food was eaten, (6) whether other factors (e.g. exercise) are necessary, and (7) the length of time since the last reaction. Any food may cause an allergic reaction, although only a few foods account for 90% of the reactions. In children these foods are eggs, milk, peanuts, soy, and wheat (fish in Scandinavian countries). In chronic disorders like atopic dermatitis, the history is often an unreliable indicator of the offending allergen.

A diet diary has frequently been used as an adjunct to the medical history. Patients are asked to keep a chronological record of all foods ingested over a specified period of time and to record any symptoms they experience during this time. The diary can then be reviewed during a patient visit to determine whether there is any relationship between the foods ingested and the symptoms experienced. Uncommonly, this method will detect an unrecognized association between a food and a patient's symptoms. But, unlike the medical history, you can collect information on a prospective basis that is not so dependent on a patient's or parent's memory.

An elimination diet is frequently used both in diagnosis and management of adverse food reactions. If a certain food or foods are suspected of provoking the reaction, they are completely eliminated from the diet. The success of an elimination diet depends on several factors, including the correct identification of the allergen(s) involved, the ability of the patient to maintain a diet completely free of all forms of the possible offending allergen, and the assumption that other factors will not provoke similar symptoms during the study period. The likelihood of all of these conditions being met is often slim. For example, in a young infant reacting to cow's milk formula, resolution of symptoms following substitution of cow's milk formula with a soy formula or casein hydrolysate (Alimentum®, Nutramigen®) is highly suggestive of cow's milk allergy, but also could be due to lactose intolerance. Avoidance of suspected food allergens before blinded challenge is recommended so the reactions may be heightened. Elimination diets, however, are rarely diagnostic of food allergy, particularly in chronic disorders such as atopic dermatitis or asthma.

Allergy skin-prick tests are highly reproducible[11] and are often used to screen patients with suspected IgE-mediated food allergies. The glycerinated food extracts (1:10 or 1:20)[9] and appropriate positive (histamine) and negative (saline) controls are applied by either the prick or puncture technique. A