

Workshop synopsis

New directions in food allergy research

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The lay public perceives "food allergy" as a major public health concern, and the US Department of Agriculture (1983) reported that 15% of the population is allergic to food.¹ Most of these reactions are nonimmunologic and more appropriately classified as "food intolerance." True food allergy, as confirmed by double-blind food challenge, occurs in far less than 15% of the population, but it is a common disease.²⁻⁵ The workshop, "New Directions in Food Allergy Research," was held to review the state-of-the-art information about food allergy and related basic research and to identify opportunities and directions for future research. The workshop reviewed clinical and epidemiologic studies of food allergy, then focused on integration of basic immunology research with clinical food allergy research. Speakers emphasized basic immunology: molecular characterization of allergens and immune mechanisms in the gastrointestinal tract, and evaluated promising new advances in research on allergic mechanisms.

FOOD ALLERGY: CLINICAL MANIFESTATIONS AND NATURAL HISTORY

Food allergy is more common in infants and young children than in adults. About 6% to 8% of infants under the age of 2 years and about 1.5% of adults in the United States are allergic to food (Sampson).^{2,3} Children are allergic to egg, milk, peanut, soy, and wheat; adults are allergic to tree nuts, peanut, fish, and crustaceans (shellfish). Sensitivity to some allergens (notably cow's milk and eggs) tends to remit in late childhood, whereas sensitivity to other allergens (e.g., peanut) often begins in infancy but does not remit. Although food-induced disease can be mediated by both IgE and non-IgE antibodies, food allergy (defined by double-blind food challenge) is mediated almost exclusively by IgE antibodies to food allergens (Metcalf). Food allergy occurs primarily in atopic patients. Atopic individuals generally have one or more atopic diseases (allergic

Abbreviations used

DBPCFC:	Double-blind placebo-controlled food challenge
HRF:	Histamine-releasing factor

rinitis, asthma, and atopic dermatitis) and a family history of these atopic diseases.

The typical symptoms of food allergy are oropharyngeal, also known as the "oral allergy syndrome," with oral and perioral itching and rash after ingestion of foods, especially fresh fruits and vegetables, and gastrointestinal (nausea, vomiting, and diarrhea); some patients have skin, respiratory, and other symptoms.^{3,4} Skin and mucosal manifestations (urticaria, erythema, angioedema) are symptoms of systemic reactions. More severe systemic signs and symptoms are respiratory and cardiovascular (dyspnea with or without wheezing, arrhythmias, hypotension). Food-induced anaphylaxis is often difficult to diagnose because cutaneous manifestations, the best way to make an early diagnosis, occur in only about 50% of such patients. Several recent studies have documented that food-induced anaphylaxis, including fatal or near-fatal reactions, is much more common than previously reported (Sampson).^{6,7} Indeed, food allergy is apparently the cause of 33% of emergency department visits for treatment of anaphylaxis and is the most frequent single cause for such visits. Peanuts and tree nuts are the foods most often associated with these severe reactions.

Another important clinical manifestation of food allergy is atopic dermatitis because expression of this skin disease is common in patients with food allergy and because food challenge can induce exacerbations of atopic dermatitis. Asthma also occurs in patients with food allergy, and patients with food allergy and asthma are at increased risk for anaphylaxis. However, in a subset of patients with food allergy who do not have asthma, food challenge results in dyspnea without wheezing, and these patients have increased bronchial reactivity.⁸

Genetic and environmental factors interact to induce food allergy. Several genetic loci have been implicated in atopic disease, including the HLA-D locus on chromosome 6p and regions on chromosomes 5q, 11q and other chromosomes (Rosenwasser). There are many possible candidate genes at these chromosomal sites, including IL-4 and IL-9 (chromosome 5) and the β chain of the high-affinity IgE receptor (Fc ϵ RI) (chromosome 11); however, these candidate genes have not been con-

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firmed. The major environmental factor appears to be allergen exposure, and both exposure levels and the age when exposed appear to be important. In infants born to atopic parents, reduction in exposure to food allergens in infancy (and in one recent study, reduction in exposure to both food and house dust mite allergens) has resulted, at age 1 year, in a reduced frequency of food allergy and diminution of some manifestations of atopy (including atopic dermatitis). A lesser reduction in atopy was observed in these children when evaluated at the ages of 2 and 4 years (Zeiger).⁹

The "gold standard" for a diagnosis of food allergy is not a specific laboratory test, but double-blind placebo-controlled food challenges (DBPCFCs) (Metcalf, Sampson). DBPCFCs support the diagnosis in only 30% to 40% of patients with a history of food allergy. Although IgE antibody to food allergens is essential for the development of food allergy, more than half of the patients with positive IgE antibody test responses to food(s) have negative DBPCFC results. Two interesting laboratory findings have been reported in patients with food allergy: (1) peripheral blood basophils from patients with active food allergy spontaneously secrete histamine, and (2) peripheral blood mononuclear cells from patients with food allergy spontaneously secrete high levels of a molecule termed *IgE-dependent histamine-releasing factor* (HRF).¹⁰⁻¹² These laboratory results reflect high levels of activation of certain leukocytes during active disease¹³ but do not provide information about which allergens are responsible for leukocyte activation.

Treatment of food allergy is limited to identification of relevant allergens followed by avoidance of these allergens (Sampson, Burks). However, avoidance is often difficult because ingredients are not always labeled, because trace contamination occurs, and because many individuals, including the patients themselves, do not believe that food allergy is a potentially fatal disease and thus do not take seriously the concept of allergen avoidance. Immunotherapy for food allergy (i.e., repeated subcutaneous injections of progressively increasing concentrations of allergen or allergens) has been used with both standard protocols (weekly injections for a period of months) and "rush" protocols (e.g., 4 injections per day for 5 consecutive days). These immunotherapy protocols have not been proven to be beneficial.¹⁴⁻¹⁶

MOLECULAR CHARACTERIZATION OF ALLERGENS

The three major allergens of peanut have been cloned and sequenced, and the IgE-binding epitopes of these allergens have been identified (Burks).¹⁷ The major allergens of shrimp, codfish, and rice and of several inhalants have also been identified and cloned (Lehrer). Interestingly, only a minority of food-derived proteins are allergens. Structural analysis of allergens has been attempted to evaluate whether characteristic features determine allergenicity of these molecules. In many, but

not all instances, allergens from diverse sources belong to common molecular families. For example, a newly recognized family, termed *calycinins*, includes major inhalant allergens from dog, rat, mouse, and cockroach, as well as a food allergen (β -lactoglobulin) (Chapman).¹⁸ Also common to many allergens is the property of intrinsic enzyme (e.g., protease) activity. A third feature is that most or all food allergens are very stable to enzymatic digestion in the gastrointestinal tract (Astwood).¹⁹ In addition to molecular characterization, certain food allergens are now being genetically engineered. In one study a Brazil nut allergen, when expressed in soybeans, retained its allergenicity, indicating (not unexpectedly) that the allergen, and not the food with which the allergen is associated, determines allergenicity.²⁰ In another study rice plants lacking the gene encoding the major rice allergen have been produced.²¹ However, many problems must be overcome (e.g., identifying all the relevant allergens and determining that none of these allergens are important in the function of the plant; establishing effective ways to delete the genes for all the allergens) to produce genetically engineered plants with reduced allergenicity.

Suggested directions for future research focused on additional studies to determine the molecular features that determine allergenicity of foods. Antigen-specific T cells can be classified, on the basis of the cytokines produced, as T_{H1} -like (producing interferon- γ but not IL-4 or IL-5), T_{H2} -like (producing IL-4 and IL-5 but not interferon- γ), and T_{H0} -like (producing interferon- γ , IL-4, and IL-5). Food allergens stimulate IgE antibody responses, and IgE responses require T_{H2} cytokines. Thus the identification of IgE-binding epitopes of allergens may enable studies to determine whether specific epitopes preferentially stimulate T_{H2} pathways. Because current immunotherapy with food allergens is of uncertain value and may be associated with a risk of severe reactions to allergen injections, safer and more effective allergen preparations are needed. Is it possible to manipulate immune responses to allergens, by modifying epitopes and adding exogenous cytokines that block T_{H2} pathways, to inhibit IgE responses? Finally, does genetic engineering offer practical approaches to modulate food allergy?

IMMUNE RESPONSES IN THE GASTROINTESTINAL TRACT

Immune responses have been characterized best in peripheral lymphoid tissue, and these appear to be different from immune responses occurring in the gastrointestinal tract. Much of the natural immune response is directed to the endogenous bacterial flora in the gastrointestinal tract. A rodent model of immunologically mediated inflammation in the intestine is entirely dependent on responses to bacterial flora, and inflammation in the intestine is entirely dependent on responses to bacterial flora, and inflammation does not occur in germ-free rodents (Cebra, Targan). Immune responses in the rodent gastrointestinal tract are self-

limited, and repeated challenge with certain antigens results in a diminished immune response, indicating that potent regulatory pathways may be present in the gastrointestinal tract. Oral administration of both high- and low-dose antigen results in a phenomenon termed *oral tolerance*, in which the immune response to subsequent systemic administration of antigen is blocked (Whitacre, Mayer).²² At least two mechanisms are operative. Tolerance to high-dose antigen is achieved by inactivation or deletion of T cells. Tolerance to low doses of antigen is achieved by stimulation of T_{H2} -like cytokines, with transforming growth factor- β being the major suppressive cytokine in several models; these cytokines inhibit the systemically induced T_{H1} response.

Directions for future research include development of better models of immunologic bowel inflammation; molecular evaluation of the mechanisms of intestinal immunity and tolerance, including the use of appropriate transgenic and gene knockout animals and/or use of germ-free and antigen-free animals; and studies of human intestinal immunity. Experiments should identify which of the many components of the intestinal immune response are of primary importance: the route of antigen presentation and the roles of different antigen-presenting cells, T-cell homing to the intestine, distinct T-cell populations, and allergen epitopes. Does the chronic low-grade inflammatory response to bacterial antigens modulate the intestinal immune response? Why do patients with food allergy have allergic responses when the normal immune response to oral antigen appears to be oral tolerance? Is an IgE immune response normal? What is the mechanism by which patients with food allergy respond to food allergens differently from non-allergic individuals? Why do allergic responses to some foods disappear in childhood, whereas allergic responses to other foods persist? Can IgE tolerance to food allergens be induced by specific immune manipulation, such as combinations of cytokines?

NEW ADVANCES IN ALLERGY RESEARCH

The workshop then focused on a number of exciting new advances in allergic mechanisms and whether such findings could be applied to food allergic disease.

One advance is in understanding the molecular nature of IgE-dependent HRF, a molecule that appears to be an important proinflammatory molecule in allergic reactions (MacDonald).¹² It appears to be particularly relevant to the pathogenesis of food allergy, because HRF production (at least in vitro) is markedly enhanced during active food allergy.¹¹ Because HRF has recently been cloned and sequenced, it is likely that antagonists of this molecule, which may be valuable in food allergy, will be developed.

Two advances may be directly relevant to the difficult problem of developing immune interventions to treat food allergy. The first of these advances concerns the recent discovery of a negative signal transduction pathway in immune and inflammatory cells, including human basophils and rodent mast cells. This negative pathway in mast cells and basophils acts by inhibiting activating

signals that are produced by cross-linking of IgE receptors; the negative pathway is activated by immune complexes that cross-link mast cell IgE and IgG receptors. Immunotherapy in allergic diseases stimulates IgG antibody production. The IgG antibodies bind allergens, and the resulting immune complexes bind IgE and IgG receptors and thus activate the negative signaling pathway (Daeron).²³ The second advance related to immunotherapy is based on DNA immunization. Plasmids incorporating coding sequences for allergen stimulate an antigen-specific, relatively "pure," T_{H1} response, which inhibits further IgE production (Raz).^{24, 25} These plasmids may also stimulate IgG production and thus activate the negative signal transduction pathway. These advances indicate the feasibility of developing effective and safe immunotherapy for food allergy.

An additional advance concerns the use of a specifically engineered, humanized anti-IgE antibody to treat allergy (Jardieu).²⁶ This anti-IgE antibody reacts only with a specific epitope of IgE, which is exposed on IgE-forming B cells, but not on IgE on the surface of basophils and mast cells. Preliminary evidence of clinical effectiveness and potent biologic activity in several model systems makes this molecule a potentially important agent for treating allergy.

A final advance is in understanding the genetics of allergic disease (Rosenwasser). Patients with food allergy are atopic, and because atopy has a major genetic component, genetic studies are highly relevant to food allergy. A number of candidate genes have been identified. It is likely that multiple genes determine allergy and that different atopic individuals have distinct genetic abnormalities to induce the atopic state. Once the abnormal genes are isolated, those responsible for food allergy will be identified. In addition, on the basis of each individual's genetic predisposition to atopic diseases, specific blockade of the abnormal gene product or products will become feasible.

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APPENDIX

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