Future Approaches to Food Allergy

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ABSTRACT. Food allergy affects ~2% of the general US population, and its prevalence seems to be increasing. Despite the potential for a fatal outcome, no definitive therapies are available for food allergy. This article reviews novel approaches for the diagnosis and treatment of food allergy. Improved diagnostic methods include more precise in vitro and in vivo tests for immunoglobulin E-mediated food allergies, in vitro assays for predicting development of oral tolerance, and novel noninvasive tests for cell-mediated food allergies such as patch testing, cytokine assays, and detection of eosinophil activation markers. Several promising novel immunomodulatory approaches to food allergy are discussed, including monoclonal anti-immunoglobulin E; probiotics; traditional Chinese medicine; and immunotherapy with modified food proteins, peptides, bacterial adjuvants, and immunostimulatory sequences. Pediatrics 2003;111:1672–1680; food allergy, diagnosis, immunomodulatory therapy, probiotics, anti-IgE antibodies, immunotherapy, traditional Chinese medicine, patch testing.

ABBREVIATIONS. IgE, immunoglobulin E; IL, interleukin; IFN-γ, γ-interferon; TNF, tumor necrosis factor; TGF-β, tumor growth factor-β; TCM, traditional Chinese medicine; FAHF-1, food allergy herbal formula-1; HKL, heat-killed Listeria; ISS-ODN, oligodeoxynucleotide immunostimulatory sequences.

Food allergy affects ~2% of the general US population, and its prevalence seems to be increasing in concert with the increasing prevalence of the extrinsic asthma and environmental allergies.1,2 However, unlike other allergic diseases, food allergy is the only disorder for which there is no specific therapy, which is particularly troubling considering the potential for severe reactions. In fact, food-induced anaphylaxis is the single most common cause of anaphylaxis evaluated in emergency departments in the United States and the United Kingdom.3,4 Dietary avoidance is the current standard of care, but accidental ingestions of food allergens are common because of the ubiquitous presence of certain foods, such as peanut, soy, milk, and egg, and compounded by poor labeling practices and cross-contamination during processing. Furthermore, in cases of multiple food allergies, restricted diets may result in unbalanced nutrition and pose a considerable hardship to the family of a food-allergic child. Therefore, accurate diagnosis of food allergy is of utmost importance and currently relies on oral food challenges, which are not without substantial risks. Considering the severity of food allergy as well as its increasing prevalence, improved diagnostic tests and definitive therapies are desirable. This article reviews the recent developments in the diagnosis and potential treatments for food allergy.

NOVEL APPROACHES TO THE DIAGNOSIS OF FOOD ALLERGY

Food-allergic disorders can be classified according to the pathophysiological role of immunoglobulin E (IgE) antibodies: immediate; IgE-mediated food allergy (eg, peanut anaphylaxis); mixed mechanisms, involving both IgE- and cell-mediated reactions (eg, atopic dermatitis, allergic eosinophilic gastroenteritis); and delayed onset, cell-mediated food allergy (eg, food protein-induced enterocolitis syndrome). Currently available diagnostic tests detect food-specific IgE, either in the skin (prick skin test) or in the systemic circulation (serum IgE levels), but there are no standard tests for the diagnosis of non–IgE-mediated food allergies. New approaches to the diagnosis of food allergy are aimed toward 3 areas: better characterization of the clinical correlation of allergenspecific IgE levels and prick skin tests, improved in vitro assays for predicting the development of clinical tolerance in IgE-mediated food allergy, and the creation of in vivo and in vitro techniques for evaluating cell-mediated food allergies (Table 1).

Improved Interpretation of Food-Specific IgE Levels

Double-blind, placebo-controlled food challenges are the gold standard for diagnosis of food allergy; however, they are labor-intensive and carry risks to the patient. A number of studies have correlated serum food-specific IgE antibody levels (measured by CAP-System Fluorescent Enzyme Immunoassay; Pharmacia-Upjohn Diagnostics, Uppsala, Sweden) to oral food challenge outcomes. Diagnostic decision points of 95% or greater positive predictive value for clinical reactivity to milk (15 kU/L), egg (7 kU/L), peanut (14 kU/L), and fish (20 kU/L) were established in a recent prospective study by Sampson,5 in which 100 children (median age: 3.8 years) were evaluated. Food-specific IgE levels exceeding the “diagnostic decision point values” indicate that the patient is >95% likely to experience an allergic reaction after the ingestion of the specific food, therefore eliminating the need to perform the oral food challenge. Detectable food-specific IgE levels that are
lower than the diagnostic decision point value but >0.35 kU/L (lower limit of detection of the CAP immunosay) represent a decreasing risk with decreasing levels of food-specific IgE. Their clinical significance requires ultimate determination by an oral food challenge. Several studies observed that in infants and young children, lower food-specific IgE levels were associated with positive food challenge outcomes, suggesting that lower IgE levels have greater positive predictive value under the age of 2 years. For egg allergy, a level of 2 kU/L or above in infants younger than 2 years had 95% positive predictive value. For milk, a level of 5 kU/L in infants younger than 1 year had 95% positive predictive value.

### In Vitro Assays for Predicting the Development of Clinical Tolerance in IgE-Mediated Food Allergy

The majority of children outgrow allergies to foods such as milk, egg, soy, and wheat; in fact 75% to 80% of milk-allergic children become clinically tolerant to milk by 4 years of age. Currently available diagnostic methods for food allergy, such as prick skin tests and serum food allergen-specific IgE levels, do not distinguish between children who will achieve food tolerance and those who will have persistent food allergy. Previous studies aimed at the identification of markers for tolerance showed that children with long-lasting milk allergy, compared with those who outgrew the allergy, have higher levels of milk-specific IgE and higher specific IgE to milk proteins including casein- and β-lactoglobulin.

IgE binding sites (epitopes) on the allergenic protein may consist of segments of consecutive amino acids (so-called sequential epitopes) or amino acids from different parts of the protein sequence, brought together by protein folding (nonsequential epitopes; Fig 1). A study by Cook and Sampson suggested that egg-allergic children who developed significant amounts of IgE antibodies to “sequential” epitopes...
of ovomucoid (major allergen in egg) were more likely to have persistent egg allergy, whereas the children who developed predominantly IgE to "non-sequential" epitopes were more likely to outgrow their egg hypersensitivity (Fig 1). Subsequent studies confirmed that ~50% of children who reacted to freeze-dried egg white (conformational nonsequential epitopes preserved) tolerated cooked egg white (conformational nonsequential epitopes destroyed by high temperature). It was recently demonstrated that children with persistent IgE-mediated milk allergy have higher levels of IgE to sequential epitopes from \( \alpha_\text{s1}, \alpha_\text{s2}, \) and \( \kappa \)-casein and that specific IgE binding to particular sequential epitopes in \( \alpha_\text{s1} \)-casein may be a predictive factor for persistence of cow milk allergy. These observations suggest that perhaps differential epitope recognition is a general feature of food allergy and additional studies should elucidate the diagnostic utility of tests that detect binding to conformational and sequential epitopes.

Recombinant Allergens

Many allergenic proteins have been identified, sequenced, and cloned. The recombinant allergens are being investigated currently for their applicability in the diagnosis of allergic diseases. Recombinant allergens of high purity offer superior safety and specificity in allergy testing, although diagnostic sensitivity is generally lower than that of natural allergen extracts. Recombinant allergens may be of special value in diagnosing allergy to plant foods in subjects with allergy to pollens. For instance, pure recombinant lipid transfer proteins could identify patients at higher risk for severe systemic reactions among the patients whose symptoms are likely to remain confined to the oropharyngeal mucous membranes (pollen-food allergy syndrome or oral allergy syndrome).

**DIAGNOSIS OF NON–IgE-MEDIATED FOOD ALLERGY**

A growing body of evidence supports the crucial role of T lymphocytes in the pathophysiology of non–IgE-mediated food allergy. As the majority of the non–IgE-mediated food-allergic disorders affect the gastrointestinal tract, endoscopy and biopsy are the requisite diagnostic tools for the detection of inflammatory cells or their products. For obvious reasons, noninvasive diagnostic methods are desired to assist in a tedious process of identifying the offending foods through elimination diets and challenge procedures. Novel approaches to non–IgE-mediated food allergy include food allergy patch testing, measurement of cytokine production by T lymphocytes after stimulation with food allergen, and measurement of cytokines and eosinophil markers in the stool. It should be noted that none of these diagnostic methods has been evaluated rigorously, and their clinical applicability remains to be determined in future studies.

**Patch Testing**

Patch testing is typically used for diagnosis of delayed contact hypersensitivity reactions in which T cells play a prominent role. Patch testing involves prolonged contact of the allergenic extract with intact
skin under occlusion for 48 hours. The results are evaluated 20 minutes after removing the patch and again at 72 hours for final evaluation. Positive reactions to patch tests consist of erythema and induration. Patch testing for the diagnosis of food allergy in children with atopic dermatitis and allergic eosinophilic esophagitis has been investigated in a number of studies. In young children with challenge-proven milk allergy, prick skin tests were positive in 67% of the cases with acute-onset reactions (under 2 hours) to milk challenge, whereas patch tests tended to be negative. Patch tests were positive in 89% of children with delayed-onset reactions (25–44 hours), although prick skin tests were frequently negative. In another study of children with atopic dermatitis, the combination of a positive patch test with evidence of specific IgE or with positive prick skin test had the highest positive predictive value. These results indicate that the combination of patch testing and detection of IgE could enhance the accuracy of diagnosing food allergy and may eliminate the need for oral food challenges. However, at this time, atopy patch testing remains an investigational method. Before incorporating atopy patch testing into clinical practice, standardization of the reagents, timing of reading results, and scoring system for the interpretation of the results is necessary.

In Vitro Assays for Lymphocyte and Eosinophil Activation

The in vitro tests for lymphocyte and eosinophil activation have been investigated in a number of studies. In general, lymphocyte proliferation assays have been proved not to be helpful in distinguishing between children with and without food allergy because they primarily reflect exposure to the food in the diet. However, the profile of cytokines produced by lymphocytes has been shown to differ in food-allergic children, producing more Th2-type cytokines (eg, interleukin [IL]-4), compared with nonallergic children, producing predominantly Th1-cytokines (eg, γ-interferon [IFN-γ]), after exposure to food allergens. In milk-allergic children with gastrointestinal symptoms, peripheral blood lymphocytes were shown to produce significantly more tumor necrosis factor (TNF-α) on stimulation with milk than in nonallergic children. As children with food allergy and atopic dermatitis frequently have chronic allergic inflammation in their gastrointestinal tract, it was hypothesized that acute-phase proteins, proinflammatory cytokines, or eosinophil secretory proteins in the stool might be increased and reflect the severity of the intestinal inflammation. Indeed, in some studies, increased amounts of fecal α1-antitrypsin, TNF-α, and eosinophil cationic protein were recovered in children with atopic dermatitis and food allergy compared with children without atopic dermatitis. These findings suggest that such noninvasive tests may be of value in monitoring the severity of intestinal inflammation in children with food allergy; however, more studies are necessary to establish the usefulness of this approach.

IMMUNOMODULATORY THERAPIES FOR FOOD ALLERGY

As first noted in murine studies, the Th1/Th2 paradigm is applied to human allergic diseases. It postulates that the type of immune response generated to an antigen depends on the profile of cytokines released by CD4+ T-helper cells (Fig 2). Typical Th1
cytokines are IFN-γ and tumor necrosis factor-β (TNF-β), whereas Th2 cytokines include IL-4, IL-5, and IL-13. In addition, regulatory cytokines such as IL-12, IL-10, and TGF-β are of paramount importance in maintaining the state of dynamic balance between Th1 and Th2 responses. Allergic diseases are characterized by relative predominance of Th2-type responses to innocuous allergens. Most of the immunomodulatory therapeutic approaches to food allergy operate on the premise of restoring the Th1/Th2 balance or activating regulatory T lymphocytes (Fig 2).

In conventional allergen immunotherapy for allergic rhinitis and asthma, gradually increasing doses of allergen are injected subcutaneously. This results in decreased symptoms attributable to a variety of immunologic alterations, including a decline in allergen-specific IgE levels, an increase in allergen-specific IgG4 antibodies, decreased basophil histamine release, decreased lymphocyte proliferation, generation of allergen-specific suppressor T cells, increased production of IFN-γ, and decreased production of Th2 cytokines such as IL-4 and TNF-β. Conventional subcutaneous allergen immunotherapy has been attempted for peanut allergy. In a double-blind, placebo-controlled trial of rush (rapidly increasing doses) peanut immunotherapy, increased tolerance to oral feeding with peanut was observed in 4 of 6 patients who received the active immunotherapy but in none of the 6 control patients. However, the rate of serious adverse reactions was unacceptably high, even during the maintenance phase of immunotherapy (39%). This important clinical study suggested that immunomodulation could be used successfully to induce oral tolerance in food-allergic subjects if activation of mast cells by allergen were eliminated and safety were improved.

Better characterization of allergen processing, presentation, and binding led to development of new immunomodulatory approaches to food allergy. In addition, characterization and cloning of major peanut allergens (Ara h1, Ara h2, and Ara h3) has made recombinant protein- and DNA-based peanut allergen gene therapy possible. Finally, testing of the investigational therapies for food allergy in vivo became possible when well-characterized mouse models of peanut and cow milk anaphylaxis were established (Fig 3).32,33

**Humanized Monoclonal Anti-IgE**

Allergen-specific IgE antibodies play a central role in the pathophysiology of atopic disorders, including food allergy, and are an excellent target to interrupt the allergic process. IgE antibodies bind to high-affinity receptors (FcεRI) on the surface of mast cells and basophils. Cross-linking of IgE molecules on the surface of mast cells by allergen leads to the release of preformed mast-cell mediators (early phase of allergic reaction) as well as synthesis of proinflammatory cytokines and chemokines that result in a late-phase reaction (Fig 4).34 Humanized monoclonal anti-IgE antibodies have been engineered to disrupt this process. They bind to the constant region (third domain of the Fc region) of IgE molecules, the point at which IgE binds to the FcεRI. This prevents IgE from binding to both high- (FcεRI) and low-affinity (FcεRII) IgE receptors. With the decrease in available IgE antibody molecules, anti-IgE leads to a down-regulation of FcεRI receptor expression on mast cells and basophils and to decreased histamine release.35 In clinical trials of anti-IgE for the treatment of asthma and allergic rhinitis, symptomatic improvement was observed when circulating levels of IgE antibodies were significantly reduced.36,37 A multicenter, randomized, double-blind, placebo-controlled clinical trial recently evaluated humanized monoclonal anti-IgE antibody in the treatment of peanut anaphylaxis. Patients with peanut allergy were orally challenged with peanut protein (double-blind, placebo-controlled food challenge) at the entry and after 4 monthly subcutaneous injections of anti-IgE or placebo to determine the amount of peanut protein required to induce anaphylaxis. Preliminary reports indicate that anti-IgE therapy significantly increased the amount of peanut protein necessary to induce allergic symptoms. If confirmed, then anti-IgE may be used to treat patients with any IgE-mediated food allergy, although the protection
against anaphylaxis would require continued therapy at regular time intervals indefinitely.

Traditional Chinese Medicine

Traditional Chinese medicine (TCM) has been used successfully in Asia for centuries for treatment of diverse diseases, including asthma and environmental allergies. TCM, based on herbal remedies, is generating increased interest because of its reported effectiveness, favorable safety profile, and low cost. Despite extensive clinical experience with TCM in Asia, the mechanism of action is largely unknown and TCM has not been studied rigorously in randomized clinical trials. Li et al investigated the effect of TCM in a murine model of allergic asthma and showed reversal of allergic airway hyperreactivity. Recently, the same group used a mixture of several herbs, designated food allergy herbal formula-1 (FAHF-1), for the treatment of peanut allergy in mice. FAHF-1 significantly reduced mast-cell degranulation and histamine release and completely prevented peanut anaphylaxis. FAHF-1 decreased peanut-IgE levels; peanut-induced lymphocyte proliferation; and IL-4, IL-5, and IL-13 production, whereas it did not affect IFN-γ synthesis. Importantly, FAHF-1 was well tolerated and had no apparent toxic effects on kidney or liver function.

Mutated Allergen Protein Immunotherapy

The major safety concern regarding food allergen immunotherapy has been addressed by engineering "hypoallergenic" forms of major allergenic food proteins. These mutated ("engineered") major food proteins have lost their ability to bind to IgE but retained the ability to interact with T cells. The 3 major peanut proteins (Ara h1, Ara h2, and Ara h3) were isolated and purified; IgE-binding and T-cell epitopes were mapped; and the DNA encoding these proteins was isolated, sequenced, and cloned. Engineered DNA encodes proteins that differ by a single amino acid within each of IgE-binding epitopes (Fig 5A). The engineered recombinant proteins bind little IgE antibodies from peanut-allergic patients but promote T-cell proliferation that is comparable to native peanut proteins. In vivo efficacy of the engineered recombinant proteins was tested in the murine model of peanut anaphylaxis. Mice were sensitized to whole peanut and then desensitized by both subcutaneous and intranasal administration of modified Ara h2 (3 doses a week for 4 weeks). Desensitization with the modified Ara h2 protein suppressed synthesis of Ara h2-IgE and resulted in significantly decreased symptoms on oral peanut challenge compared with a control group treated with native Ara h2.

Bacterial adjuvants are being used to increase efficacy of modified peanut vaccines. Bacteria are potent stimulants of Th1 immune responses and increase IFN-γ production. Heat-killed Listeria (HKL) has been shown to reverse established allergic airway hyperreactivity in mice. In a dog model, a single subcutaneous treatment with a mixture of HKL, milk, and wheat significantly reduced immediate skin test reactions and prevented anaphylactic symptoms after oral food challenge. Li et al recently tested the efficacy of a mixture of HKL and modified peanut proteins (Ara h1, Ara h2, and Ara h3) in a murine model of peanut anaphylaxis. Immunotherapy was initiated 10 weeks after sensitization (once a week for 3 weeks) and was shown to reduce peanut-
specific IgE synthesis as well as anaphylactic responses to oral peanut challenge. Heat-killed *Escherichia coli* expressing modified peanut proteins is being investigated in the same model. Although lower doses for desensitization were required using HKL and Heat-killed *E coli*, the safety of these therapeutic approaches need additional evaluation.

**Peptide Immunotherapy**

The effect of eliminating IgE binding can also be achieved with vaccines that consist of overlapping peptides (10–20 amino acids long) that represent the entire sequence of a specific protein (Fig 5B). The antigen-presenting cells are provided with all possible T-cell epitopes, but mast cells are not activated because the short peptides are of insufficient length to cross-link 2 IgE molecules. Peptide immunotherapy allows for formulation of vaccines against any food in which major allergenic proteins are known because IgE binding sites for each food protein do not have to be mapped, allergen-specific DNA does not need to be mutated, and engineered recombinant proteins are not required. Major peanut protein Ara h2 peptide mixture is currently being evaluated in a mouse model of peanut allergy. Pretreatment with 2 doses of the major peanut protein Ara h2 peptide mixture before peanut challenge has been shown to prevent anaphylactic reactions in peanut-sensitized mice. More extensive desensitization protocols are being investigated.

**Immunostimulatory Sequences**

Certain portions of bacterial DNA designated as oligodeoxynucleotide immunostimulatory sequences (ISS-ODN) are potent stimulators of Th1 responses. These bacterial DNA components activate antigen-presenting cells, natural killer cells, and B cells and upregulate Th1 cytokine production (eg, IFN-γ). ISS-ODNs have been shown to prevent airway allergic inflammation in the murine models of asthma. Li and colleagues studied the prophylactic use of ISS-conjugated peanut protein Ara h2 in mice later sensitized with peanut allergen. Mice were immunized intradermally with ISS-conjugated Ara h2 or control ISS. Four weeks later, mice were orally sensitized with peanut and challenged with Ara h2 5 weeks later. Mice that were immunized with ISS-Ara h2 control ISS. Four weeks later, mice were orally sensitized with peanut and challenged with Ara h2 5 weeks later. Mice that were immunized with ISS-Ara h2 conjugate did not develop severe anaphylactic symptoms after peanut challenge, and their postchallenge plasma histamine levels were significantly lower than in sham-treated mice, which developed anaphylactic symptoms. These data suggest that antigen ISS-ODN immunotherapy may prevent the development of food allergy; however, its potential to

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Fig 5. Modified recombinant allergens and peptide immunotherapy. A, Modified recombinant allergen. The substitution of a single amino acid within the IgE-binding epitope results in abrogation of IgE binding. B, Peptide immunotherapy. The sequence of a protein is represented by the overlapping peptides (10–20 amino acids long) that provide all possible T-cell epitopes, but binding to IgE antibody is eliminated.
reverse established food allergy remains to be determined.

Probiotics

Probiotics are live bacteria or their components that are reported to have beneficial effect on the health of the host by improving its intestinal microbrial balance. The major sources of probiotics are dairy products that contain *Lactobacillus* and *Bifidobacterium* species. Although the concept of probiotic foods was introduced more than a century ago by Fuller, only recently were probiotics evaluated by randomized, double-blind, controlled clinical trials in the treatment of food allergy. In infants and young children with cow milk hypersensitivity, 2-month treatment with *L rhamnosus* GG and *B lactis* along with a milk elimination diet decreased the severity of atopic dermatitis symptoms.52 Another study evaluated the prophylactic potential of probiotics. A total of 159 expectant mothers who had at least 1 first-degree relative (or partner) with history of atopic disease (atopic dermatitis, asthma, or allergic rhinitis) were randomized to receive either *Lactobacillus GG* supplementation or placebo.53 Treatment was continued during breastfeeding and also was given to the infants for 6 months, starting on the first day of life. At 2 years of age, 23% of children were reported to have atopic dermatitis in the probiotic-treated group compared with 46% in the placebo group, indicating that *Lactobacillus GG* had some effect on the prevention of early atopic disease in infants at high risk. However, this study raises several questions. The prevalence of atopic dermatitis in the placebo-treated group was considerably higher than that reported in other studies, and the severity of atopic dermatitis was very low (geometric mean SCORAD index of 10.4 out of maximum 103) and not different from the probiotic-treated group (geometric mean SCORAD index of 9.8). Furthermore, there was no difference between the 2 study groups in any of the objective markers, such as the prevalence of positive prick skin tests to selected food and environmental allergens, total serum IgE, or serum allergen-specific IgE levels to milk, egg, cat, and house dust mite.54–56

CONCLUSIONS

Improved interpretation of in vivo and in vitro tests for IgE-mediated food allergy, in vitro assays for predicting development of oral tolerance, and tests for non–IgE-mediated food allergy will facilitate diagnosis of food allergy and eliminate unnecessary oral food challenges. Novel approaches to the treatment of IgE-mediated food allergy such as anti-IgE, probiotics, and food allergy vaccines will hopefully provide definitive therapy for food-allergic patients and prevent development of food allergy in at-risk infants. However, these immunomodulatory therapies will have to be evaluated carefully for potential side effects such as toxicity, overstimulation of the TH1 immune responses, priming for autoimmunity, and long-term effects of suppression of circulating IgE antibodies. Nevertheless, these new approaches bring real hope to the patients for whom no specific therapy is currently available.

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