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Future therapies for food allergies

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Activity Objectives

1. To summarize the current management and candidate therapies for IgE-mediated forms of food allergy.
2. To understand that allergen-specific immunotherapy can include feeding patients heat-treated allergen, as well as oral, sublingual, and epicutaneous immunotherapy.
3. To recognize that allergen-nonspecific therapy might include humanized monoclonal anti-IgE antibodies, Food Allergy Herbal Formula (FAHF) 1, probiotics, and Toll-like receptor agonists.
4. To review key clinical trials and studies in food allergy treatment.

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Food allergy is an increasingly prevalent problem in westernized countries, and there is an unmet medical need for an effective form of therapy. A number of therapeutic strategies are under investigation targeting foods that most frequently provoke severe IgE-mediated anaphylactic reactions (peanut, tree nuts, and shellfish) or are most common in children, such as cow's milk and hen's egg. Approaches being pursued are both food allergen specific and nonspecific. Allergen-specific approaches include oral, sublingual, and epicutaneous immunotherapy (desensitization) with native food allergens and mutated recombinant proteins, which have decreased IgE-binding activity, coadministered within heat-killed *Escherichia coli* to generate maximum immune response. Diets containing extensively heated (baked) milk and egg represent an alternative approach to food oral immunotherapy and are already changing the paradigm of strict dietary avoidance for patients with food allergy. Nonspecific approaches include monoclonal anti-IgE antibodies, which might increase the threshold dose for food allergen in patients with food allergy, and a Chinese herbal formulation, which prevented peanut-induced anaphylaxis in a murine model and is currently being investigated in clinical trials. The variety of strategies for treating food allergy increases the likelihood of success and gives hope that accomplishing an effective therapy for food allergy is within reach. (J Allergy Clin Immunol 2011;127:558-73.)

Key words: *Food allergy, oral immunotherapy, sublingual immunotherapy, probiotics, epicutaneous immunotherapy, desensitization, milk allergy, peanut allergy, egg allergy, anti-IgE, anti-IgE therapy, anti–IL-5 therapy*

Over the past 2 decades, food allergy has emerged as a major public health problem in westernized societies.[1,2] In American children younger than 18 years, the prevalence of food allergy has increased by 18% and the prevalence of peanut allergy has tripled (0.4% to 1.4%) from 1997 to 2008.[3,4] Food allergy is the most common cause of anaphylaxis evaluated in the emergency department in all age groups, and the number of hospitalizations for food-induced anaphylaxis has increased more than 3-fold in the past decade in the United States and United Kingdom.[3,5,6] Food-induced anaphylaxis occasionally results in fatalities, with

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Abbreviations used
DBPCFC: Double-blind, placebo-controlled food challenge
EoE: Eosinophilic esophagitis
EPIT: Epicutaneous immunotherapy
FAHF: Food Allergy Herbal Formula
FoxP3: Forkhead box protein 3
HKE: Heat-killed *Escherichia coli*
HKLM: Heat-killed *Listeria moncytogenes*
ISS: Immunostimulatory sequence
Man_{51}-BSA: Mannoside-conjugated BSA
OIT: Oral immunotherapy
pDNA: Plasmid DNA
PFAS: Pollen-food allergy syndrome
SIGNR-1: C-type lectin receptor, also called CD209b
SLIT: Sublingual immunotherapy
TCM: Traditional Chinese medicine
TLR9: Toll-like receptor 9

more than 90% of deaths in the United States caused by reactions to peanut or tree nuts.[7,8]

The current management of food allergy is limited to strict dietary avoidance, nutritional counseling, and emergency treatment of adverse reactions.[9] In this review we will focus on efforts to treat IgE-mediated forms of food allergy. Although attempts to desensitize patients with food allergy date back more than 100 years, such as oral immunotherapy (OIT),[10] there are no accepted therapies proved to accelerate the development of oral tolerance or to provide effective protection from unintentional exposures.[1] However, a number of therapeutic strategies are under investigation targeting foods that most frequently provoke severe IgE-mediated anaphylactic reactions (peanut, tree nuts, and shellfish) or are most common in children, such as cow's milk and hen's egg.[11] Approaches being pursued are both food allergen specific and nonspecific (Fig 1).[12] Allergen-specific approaches include OIT, sublingual immunotherapy (SLIT), and epicutaneous immunotherapy (EPIT; desensitization) with native food allergens and mutated recombinant proteins, which have decreased IgE-binding activity, coadministered within heat-killed *Escherichia coli* (HKE) to generate maximum immune response. Diets containing extensively heated (baked) food, such as milk or egg, might represent an alternative approach to allergen-specific immunomodulation of food allergy in some patients.

Nonspecific approaches include anti-IgE mAbs, which might increase the threshold dose for reactivity to food allergens, and a Chinese herbal formulation, which prevented peanut-induced anaphylaxis in a murine model of peanut-induced anaphylaxis and is currently being investigated in clinical trials.

SELECTION OF CANDIDATES FOR NOVEL FOOD ALLERGY THERAPIES

Food allergies seriously alter the quality of life of patients with food allergy and their families. Fortunately, about 85% of children allergic to foods such as cow's milk, egg, wheat and other cereal grains, and soy "outgrow" (develop tolerance) their allergy, whereas only 15% to 20% of children allergic to peanut, tree nuts, fish, and shellfish will show spontaneous tolerance. Diagnostic tests are needed that can distinguish subjects with

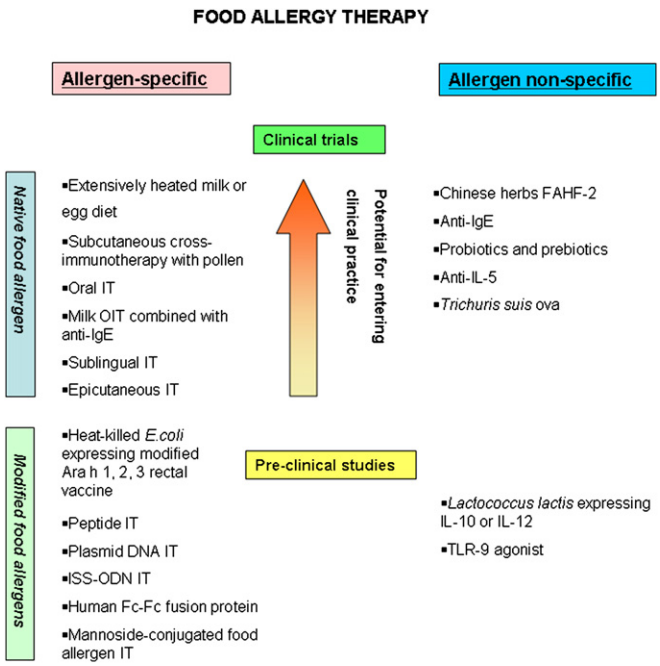

FIG 1. Approaches to food allergy immunotherapy. *ISS-ODN,* Immunostimulatory oligodeoxynucleotide; *IT,* immunotherapy.

transient from persistent forms of food allergy so that therapeutic strategies can be used early to accelerate the induction of tolerance in those who can outgrow their allergy or to induce tolerance in those with the persistent form. Currently, there are no diagnostic tests (eg, serum food allergen–specific IgE antibody measurement or skin prick tests) that reliably predict the potential for spontaneous development of oral tolerance. However, 2 recent reports in children with multiple food allergies noted that few children with peak cow's milk– or egg white–specific IgE antibody levels of 50 kU_A/L or greater (UniCAP; Phadia, Uppsala, Sweden) outgrow their allergy by their late teenage years.[13,14] In addition, recent studies using peptide microarray assays to determine the diversity and affinity of IgE binding to sequential epitopes on major food allergens (eg, peanut, cow's milk, and egg white) might be useful in determining the severity and persistence of food allergy in affected patients (Table I).[15-23]

IMMUNOTHERAPEUTIC APPROACHES FOR TREATING FOOD ALLERGY

Patients with food allergy can be divided into 3 basic phenotypes: transient food allergy, persistent food allergy, and food-pollen (oral allergy) syndrome. Based on developing evidence, it appears that each of these forms of IgE-mediated food allergy is the result of different immunologic mechanisms and therefore is likely to require different immunotherapeutic approaches to bring about resolution.

It appears that patients with transient food allergy will have the most favorable response to therapy. Although it might be argued that transient food allergy does not require treatment, the potential benefits of therapy include accelerated development of tolerance and improved quality of life and nutrition.

Persistent food allergy might present a more challenging situation. Patients with the persistent form of food allergy are

TABLE I. Significance of sequential IgE-binding epitopes in egg white, cow's milk, and peanut

	Patient population and methods	Results
Egg white ovomucoid		
Cooke and Sampson, 1997[15]	Children with persistent egg allergy and atopic dermatitis: ovomucoid dodecapeptides overlapping by 10 amino acids were synthesized on a SPOTs membrane.	Serum from a subject with transient egg allergy had no IgE antibodies against reduced and alkylated (sequential epitopes) ovomucoid, whereas serum from a subject with persistent egg allergy recognized sequential ovomucoid epitopes.
Jarvinen et al, 2007[16]	Eleven children with transient and 7 children with persistent egg allergy: the central decapeptides from each of the major IgE-binding epitopes of ovomucoid synthesized on a SPOTs membrane: Immunolabeling was done with individual patients' sera.	Both groups had comparable ranges of egg-specific IgE levels, but none of the patients with transient egg allergy had IgE antibodies against these epitopes of ovomucoid: amino acids 1-10, 11-20, 47-56, and 113-122. In contrast, all 7 patients with persistent egg allergy recognized at least 4 of these immunodominant epitopes.
Milk		
Jarvinen et al, 2001[17]	Ten patients with persistent milk allergy and 10 patients who subsequently outgrew their milk allergy: 25 decapeptides of α_{s1}-casein, α_{s2}-casein, κ-casein, α-lactalbumin, and β-lactoglobulin, comprising the core epitopes, synthesized on a SPOTs membrane. Sera from individual patients were used for immunolabeling.	Five IgE-binding epitopes (2 on α (s1)-casein, 1 on α (s2)-casein, and 2 on κ-casein) were not recognized by any of the patients with transient milk allergy but showed binding by the majority of the patients with persistent allergy. Antibodies against at least 1 of 3 epitopes (amino acids 123-132 on α_{s1}-casein, amino acids 171-180 on α_{s2}-casein, and amino acids 155-164 on κ-casein) were identified in all patients with persistent milk allergy.
Wang et al, 2010[18]	Thirty-three children with milk allergy and 8 children who outgrew milk allergy. Peptides, consisting of 20 amino acids overlapping by 17 (3-offset) and corresponding to the primary sequences of α_{s1}-, α_{s2}-, β-, and κ-caseins and β-lactoglobulin, were arrayed on glass slides.	Subjects with milk allergy had increased epitope diversity compared with those who outgrew milk allergy. Binding to higher numbers of IgE peptides was associated with more severe allergic reactions during challenge. In a competitive peptide microarray assay, allergic patients demonstrated a combination of high- and low-affinity IgE binding, whereas subjects who had outgrown their milk allergy had primarily low-affinity binding.
Peanut Ara h 1, Ara h 2, Ara h 3 (peptide microarray)		
Shreffler et al, 2004[19]	Seventy-seven patient and 15 control sera were analyzed. A set of 213 overlapping 20-residue peptides was synthesized, corresponding to the primary sequences of Ara h 1, Ara h 2, and Ara h 3. Peptides were arrayed in triplicate along with the corresponding recombinant proteins onto glass slides and used for immunolabeling.	The majority of patients (97%) had specific IgE to at least 1 of the recombinant allergens; 87% had detectable IgE to sequential epitopes. Individual patients had significant heterogeneity in the numbers and patterns of epitopes recognized. High epitope diversity was found in patients with a history of more severe allergic reactions.
Flinterman, 2008[20]	Twenty-four peanut-sensitized children and 6 atopic control subjects: specific IgE and IgG4 binding to 419 overlapping 15-amino-acid peptides representing the sequence of recombinant Ara h 1, Ara h 2, and Ara h3 was analyzed with a microarray immunoassay.	Peanut-sensitized sera bound significantly more IgE and IgG4 epitopes than control sera. There was a positive correlation between the number of IgE epitope recognized and clinical sensitivity ($r = 0.6$, $P = .021$). IgE and IgG4 epitope-recognition patterns were stable over a 20-month period.

likely to have a less favorable response to therapy, including failure to desensitize, failure to have oral tolerance, need for a more prolonged treatment course, and development of more serious adverse reactions during therapy. As experience with various treatment regimens increases, we will be better equipped to counsel patients about optimal individualized therapeutic options.

ALLERGEN-SPECIFIC IMMUNOTHERAPY

Diet containing extensively heated milk and egg

Several studies have demonstrated that children with transient egg and milk allergy produce IgE antibodies directed primarily against conformational IgE-binding epitopes that are destroyed during extensive heating or food processing.[15,16] Based on these observations, we hypothesized that children with transient milk and egg allergy, which comprises up to 80% of children with milk and egg allergy, would tolerate baked products containing milk and egg. Two clinical trials investigated the tolerance of extensively heated (baked into other products) milk and egg in children with milk and egg allergy.[24,25] In both studies approximately 80% of children tolerated extensively heated milk and egg products during an initial physician-supervised oral challenge. Severe reactions that required treatment with epinephrine occurred only in children who reacted to the extensively heated milk products but not in children who tolerated extensively heated milk and reacted to unheated milk. In contrast, there was no such distinction

in children reacting to extensively heated or unheated egg products.

Food-specific IgE levels and skin prick test responses were not reliable markers for identifying children tolerant to extensively heated milk or egg, and oral food challenges were necessary. However, the majority of children who reacted to extensively heated milk had milk-specific IgE antibody levels of greater than 35 kU_A/L (UniCAP, Phadia). In a study conducted in a different patient population, a positive decision point for reactivity to heated egg was 10.8 kU_A/L, and the negative decision point was 1.2 kU_A/L (UniCAP, Phadia).[26]

Children who reacted to extensively heated milk had significantly higher basophil reactivity to stimulation with casein compared with that seen in the extensively heated milk–tolerant children.[27] There was a significantly higher median percentage (16.9%; 25th-75th percentile, 7.1% to 31.7%) of proliferating casein-specific $CD25^+CD27^+$ T cells from casein-induced PBMC cultures of 18 extensively heated milk–tolerant subjects compared with 8 subjects reactive to extensively heated milk (4.9% [25th-75th percentile, 2.6% to 7.5%], $P < .01$).[28] There were no significant differences between the groups in the frequency of polyclonal T cells or casein-specific effector T cells. Casein-specific regulatory T cells were forkhead box protein 3 (FoxP3)–positive, $CD25^{hi}$, $CD27^+$, cytotoxic T lymphocyte–associated antigen 4–positive, $CD45RO^+$, and $CD127^-$. Depletion of the $CD25^{hi}$ cells before *in vitro* culture significantly enhanced casein-specific effector T-cell expansion, confirming the presence of greater regulatory T-cell activity. A higher frequency of casein-specific regulatory T cells correlated with a phenotype of mild transient milk allergy and favorable prognosis.

Baked goods with milk or egg were added to the diets of tolerant children, who were followed up every 3 to 6 months. No increases were seen in acute allergic reactions or in the severity of underlying atopic diseases, such as asthma, atopic dermatitis, or eczema. There was no increase in the intestinal permeability (determined with measurement of a urinary clearance of lactulose and mannitol) over the first year on the diet and no negative effects on growth. Immunologic changes observed after the introduction of baked goods with milk and egg into the diet included increasing food-specific IgG4 antibodies, decreasing wheal sizes on skin prick tests, and a trend for decreasing food-specific IgE antibody levels, findings similar to those observed in patients undergoing OIT. Preliminary findings suggest that many of the children started on baked products experience accelerated tolerance induction, and a large study is ongoing to establish the safety and efficacy of introducing baked products into the diets of tolerant children as a form of immunotherapy.

Subcutaneous peanut immunotherapy

Subcutaneous immunotherapy has been used for more than 100 years to treat environmental allergies. In a study using an aqueous extract of peanut, 3 actively treated subjects displayed a 67% to 100% decrease in symptoms during double-blind, placebo-controlled food challenges (DBPCFCs) and had a 2- to 5-log reduction in end point skin prick test reactivity to peanut at the end of the treatment course, whereas 1 placebo-treated subject had no change in these parameters.[29] As a consequence of a pharmacy error, 1 placebo-treated subject died of anaphylaxis after administration of a dose of peanut extract, resulting in the termination of the study. This tragic event highlighted the serious risks of peanut immunotherapy.

In a follow-up study 6 subjects were treated with a maintenance dose of 0.5 mL of 1:100 wt/vol peanut extract, and 6 were followed as an observational untreated control group for 12 months.[30] At the end of 12 months, all 6 treated subjects tolerated an increased peanut threshold dose during oral food challenges and had decreased sensitivity on titrated peanut skin prick tests, whereas untreated control subjects experienced no improvement in these parameters. However, anaphylaxis with respiratory involvement was provoked a mean of 7.7 times during the rush phase (23% of the doses), with an average of 9.8 epinephrine injections per subject treated with peanut immunotherapy. Only 3 of 6 subjects were able to achieve the intended maintenance dose because of frequent adverse reactions. During the maintenance phase, the rate of systemic reactions was 39%, with an average of 12.6 epinephrine injections per subject. Although this study provided evidence that injected food allergen could induce desensitization, the high rate of unpredictable severe adverse reactions discouraged further evaluation of this form of therapy.

Immunotherapy with pollen for the cross-reactive food

The concept of cross-immunotherapy has been applied to the pollen-food allergy syndrome (PFAS; also referred to as oral allergy syndrome). Several studies showed variable beneficial effects on oral symptoms and skin test reactivity to certain plant foods in subjects treated with pollen subcutaneous immunotherapy or SLIT.[31-35] An open trial of birch subcutaneous immunotherapy in 49 adults with birch-induced allergic rhinitis and PFAS to apple found a significant reduction (50% to 95%) or complete resolution of apple-induced oral symptoms in 84% of treated subjects compared with no benefit in control subjects ($P < .001$) and a reduction in skin test reactivity to fresh apple in 88% of subjects at the end of the 12-month course of birch immunotherapy.[31] In a follow-up study more than 50% of subjects tolerated apple at the 30-month visit (18 months after discontinuation of birch immunotherapy); however, the majority reverted to positive skin prick test responses. Other clinical trials in which oral allergy symptoms to apple and other raw foods were diagnosed with DBPCFCs support a beneficial effect of birch subcutaneous immunotherapy in a subset of subjects.[33,34,36] SLIT with birch pollen extract in adults with birch-induced allergic rhinitis did not significantly reduce apple-induced PFAS symptoms.[37]

The beneficial effects on PFAS were predominantly reported in adults monosensitized to birch tree pollen and treated with high-dose pollen immunotherapy. T-cell immune responses to birch pollen cross-reactive major food allergens, such as apple Mal d 1, hazelnut Cor a 1, and carrot Dau c 1, are partially Bet v 1 independent. Therefore vaccines based on modified, recombinant food allergens might represent a superior approach to the treatment of PFAS.

FOOD OIT

Successful OIT in a boy with egg-induced anaphylaxis was first reported in 1908.[10] At present, OIT to food is one of the most actively investigated therapeutic approaches for food allergy, although few trials have established patient reactivity before therapy, included a placebo control, or both. Furthermore, although studies suggest that a majority of patients with food allergy can be desensitized with OIT, no studies have demonstrated the development of tolerance. In addition, adverse reactions during therapy

TABLE II. Trials in food OIT

Study	Subjects	Success rate*	Immunologic changes	Side effects/comments
Mixed foods				
Patriarca et al, 2003,[39] clinical trial	Milk (n = 29) Egg (n = 15) Fish (n = 11) Orange (n = 2) and other†	45/54 (83.3%)	SPT responses became negative after 18 mo in 78%; food-specific IgE levels decreased and food-specific IgG4 levels increased after 18 mo.	Fifty-one percent of patients experienced urticaria, emesis, diarrhea, or abdominal pain. In 9 (16.7%) patients the protocol was stopped because of side effects. No differences between children and adults were found.
Morisset et al, 2007,[40] randomized clinical trial	N = 141 Mean age: milk, 2.2 y; egg, 3.5 y Milk (n = 57) Egg (n = 84)	Milk: 89% Egg: 69%	SPT response sizes and specific IgE levels were significantly decreased in children in whom tolerance to milk or egg developed.	Only children tolerating at least 60 mL of milk or 965 mg of raw egg white on a baseline food challenge were included. SPT sizes and specific IgE levels were significantly decreased in children in whom tolerance to milk or egg developed.
Staden et al, 2007,[38] randomized clinical trial	Milk (n = 14) Egg (n = 11) Control group (n = 20)	Nine (36%) of 25 had permanent tolerance; 3 (12%) of 25 were tolerant with regular intake (desensitized); and 4 (16%) of 25 were partial responders.	Allergen-specific IgE levels decreased significantly both in children who had natural tolerance during the elimination diet ($P < .05$) and in those treated with OIT ($P < .001$).	The first study to test the permanence of the therapeutic effect after a 2-mo period of complete avoidance of the food. The spontaneous resolution rate of food allergy in the control group was comparable (7/20 [35%]).
Cow's milk				
Meglio et al, 2004[47]	N = 21 Age: 5-10 y	15/21 (71.4%)	SPT responses to BLG and CS significantly decreased at 6 mo ($P < .001$). Milk-specific IgE levels were not significantly different.	Three of 21 reacted to the minimal dose of diluted milk. Three of 21 tolerated only 40-80 mL of milk per day. Fifteen of 21 tolerated 200 mL of milk per day for 6 mo. The side effect rate was 13 of 21.
Skripak et al, 2008,[41] randomized, placebo-controlled clinical trial; Narisety et al, 2009,[42] open-label follow-up study	N = 20; Active/placebo ratio, 2:1; age, 6-17 y	Nineteen subjects completed treatment. After OIT, the median cumulative dose of milk inducing a reaction in the active group increased from 40 mg to the median dose of 5,140 mg. There was no change in the placebo group ($P = .0003$).	Milk-specific IgE levels did not change significantly in either group. Milk-specific IgG levels increased significantly in the active treatment group, with a predominant milk-specific IgG4 increase.	The median frequency of side effects was 35% in the active group compared with 1% in the placebo group. *Blinded study*: Mild oral pruritus, median 16% doses per child Gastrointestinal, median 2% doses/child Epinephrine, 0.2% of total doses; 2 doses during build-up and 2 doses during home maintenance (in 4 subjects) *Open-label home study*: 1-3 mo, 2.5% to 96.4% of doses per subject; >3 mo: 0% to 79% per subject Percentage of total doses with reactions: Oral pruritus, 17% Gastrointestinal, 3.7% Respiratory, 0.9% Cutaneous, 0.8% Multisystem, 5.5% Epinephrine, 6 reactions in 4 subjects

(Continued)

TABLE II. *(Continued)*

Study	Subjects	Success rate*	Immunologic changes	Side effects/comments
Longo et al, 2008,[43] randomized clinical trial	N = 60; active treatment, n = 30; untreated comparison, n = 30 Mean age, 7.9 y (5-17 y)	After 1 y, 11 (36%) of 30 tolerated ≥150 mL of milk on a daily basis; 16 of 30 tolerated 5-150 mL of milk. None of the children in the comparison group tolerated >150 mL of milk during the final food challenge ($P < .001$).	Reduction in cow's milk–specific IgE in 15 of 30 subjects treated with milk OIT; no clear changes in the untreated group comparison subjects	Three (10%) children discontinued the study because of significant respiratory or abdominal side effects. Seventeen of 30 children reported side effects at home. Seventeen children received oral steroids, 6 received nebulized epinephrine, and 1 received intramuscular epinephrine for home reactions. Six of the comparison subjects had mild symptoms on accidental exposures to milk during the study.
Peanut				
Jones et al, 2009[44]	N = 39 Median age at enrollment: 57.5 mo (range, 12-111 mo)	Open-label study, follow-up for 30 mo: 29 (74%) of 39 subjects complete; 27 (77%) of 35 subjects ingested 3.9 g of peanut protein during the final food challenge.	By 6 mo, titrated skin prick test responses and activation of basophils significantly decreased. Peanut-specific IgE levels decreased by 12-18 mo, whereas IgG4 levels increased significantly. Serum factors inhibited IgE-peanut complex formation in an IgE-facilitated allergen-binding assay. Secretion of IL-10, IL-5, IFN-γ, and TNF-α from PBMCs increased over a period of 6-12 mo. Peanut-specific $FoxP3^+$ T cell numbers increased until 12 mo and decreased thereafter. T-cell microarrays showed downregulation of genes in apoptotic pathways.	Four (10%) subjects withdrew because of side effects. Six subjects withdrew because of personal reasons. Most symptoms noted during OIT resolved. spontaneously or with antihistamines. Adverse Reactions during the *build-up phase* (% of total doses)[39,41,42]: Mild oropharyngeal, 69% Mild-to-moderate skin, 62% Mild-to-moderate nausea or abdominal pain, 44% Diarrhea/emesis, 21% Mild wheezing, 18% Adverse reactions during the *maintenance phase* (% of total doses): Upper respiratory, 29% Cutaneous, 24% Any treatment, 0.7% of home doses Epinephrine, 2 subjects (1 dose each)
Clark et al, 2009[48]	N = 4 Case series: ages 9-13 y	Open-label study: follow-up for 6 wk on maintenance dose of 800 mg of peanut flour. All subjects tolerated significantly more peanut flour during a final open peanut challenge than during the baseline DBPCFC.	No information.	Peanut OIT was well tolerated; no epinephrine was used for treatment of adverse reactions.

(Continued)

TABLE II. *(Continued)*

Study	Subjects	Success rate*	Immunologic changes	Side effects/comments
Blumchen et al, 2010[46]	N = 23; Age, 3.2-14.3 y	After the rush phase, patients tolerated a median of 0.15 g of peanut. Twenty-two of 23 patients continued with the long-term protocol. After a median of 7 months, 14 (63%) patients reached the protective dose of 0.5-2 g of peanut. At the final DBPCFC, patients tolerated a median of 1 g (range, 0.3-4 g) in comparison with 0.2 g peanut at the DBPCFC before OIT (range, 0.02-1 g; $P = .002$).	There was a significant increase in peanut-specific serum IgG4 levels and a decrease in peanut-specific IL-5, IL-4, and IL-2 production by PBMCs after OIT.	In 2.6% of 6,137 total daily doses, mild-to-moderate side effects were observed; in 1.3% lower respiratory tract symptoms occurred. OIT was discontinued in 4 (18%) of 22 patients because of adverse events. No epinephrine was used for treatment of adverse reactions.
Egg				
Buchanan et al, 2007[48]	N = 7 Mean age, 4 y (subjects with history of egg-induced anaphylaxis were excluded)	4/7 (57%)	Egg white–specific IgG levels increased significantly from baseline to 24 mo ($P = .002$). Five subjects showed an overall decrease in egg white–specific IgE levels.	Four subjects tolerated egg challenge at the end of 24 mo. Two of them reacted to a subsequent egg challenge done 3 mo after treatment was stopped.

BLG, β-Lactoglobulin; *CS*, casein; *SPT*, skin prick test.
*Success rate is defined as regular ingestion of the tested food for at least 6 months.
†One each of apple, peach, lettuce, orange, beans, and corn.

are common (ie, >25% of doses associated with adverse symptoms, although most are mild in nature; Table II).[38-48]

Oral tolerance versus desensitization

The ultimate goal of food allergy therapy is permanent oral tolerance, which is established when the food can be ingested without allergic symptoms despite prolonged periods of avoidance. The mechanism of permanent oral tolerance likely involves the initial development of regulatory T cells and immunologic deviation away from the proallergic T_H2 response, followed by anergy at later stages.[49] In contrast, in a "desensitized state" protection depends on the regular ingestion of the food allergen; when dosing is interrupted or discontinued, the protective effect might be lost or significantly decreased. Immunologic changes accompanying oral desensitization include decreased reactivity of mast cells and basophils, increased food-specific IgG4 antibodies, and eventually decreased food-specific IgE antibodies. The permanence of protection can be tested with intentional interruption of dosing for at least 4 to 12 weeks followed by a supervised oral food challenge.[38,50]

Dosing schedule

During OIT, food is mixed in a vehicle (safe food) and ingested in gradually increasing doses. Dose escalation typically occurs in a controlled setting, and daily regular ingestion of tolerated doses during the build-up and maintenance phases occurs at home. Early uncontrolled studies provided evidence that a subset of patients with food allergy could be "desensitized" to a variety of foods, including milk, egg, fish, fruit, peanut, and celery.[39,40,51,52] Some subjects who tolerated a maintenance dose, even for a significant period of time, had allergic symptoms again if the food was not ingested on a regular basis, highlighting a concern that permanent tolerance was not achieved.[50]

Milk OIT

In a large trial of OIT, 45 (median age, 2.5 years; range, 0.6-12.9 years) children with challenge-proved, IgE-mediated cow's milk or egg allergy were randomly assigned to OIT (n = 25) or an elimination diet as a control group (n = 20).[38] OIT with fresh cow's milk or lyophilized egg protein was given at home daily. After a median of 21 months, children in the OIT group were started on an elimination diet for 2 months before a follow-up rechallenge to determine whether oral tolerance had been achieved. At the follow-up challenge, there was no difference in the rate of tolerance between the 2 groups: 9 (36%) of 25 children receiving OIT had permanent tolerance compared with 7 (35%) of 20 control children. Allergen-specific IgE levels decreased significantly in children with natural tolerance during the elimination diet ($P < .05$), as well as in those treated with OIT ($P < .001$). In addition, 3 (12%) children in the OIT group could tolerate milk and 4 (16%) children could tolerate increased amounts of milk/egg compared with baseline during active therapy.

In the first randomized, double-blind, placebo-controlled trial of OIT, 20 children with IgE-mediated milk allergy were randomized to milk or placebo OIT.[41] Dosing occurred in 3 phases: the build-up in-office day (initial dose, 0.4 mg of milk protein; final dose, 50 mg), daily doses with 8 weekly in-office dose increases to a maximum of 500 mg, and continued daily maintenance doses at home for 3 to 4 months. Nineteen patients, 6 to 17 years of age, completed the treatment: 12 in the active group and 7 in the placebo group. The median milk threshold dose in both groups was 40 mg at the baseline DBPCFC. After OIT, the median threshold dose in the active treatment group was 5,140 mg (range,

2,540-8,140 mg), whereas all patients in the placebo group only tolerated 40 mg ($P = .0003$). All children in the active treatment group experienced adverse reactions because of OIT. Among 2,437 active OIT doses and 1,193 placebo doses, there were 1,107 (45.4%) and 134 (11.2%) adverse reactions, respectively, with local symptoms being most common. Milk-specific IgE levels did not change significantly in either group. Milk IgG4 levels increased significantly in the active treatment group. In a follow-up, open-label study 15 children (6-16 years old) were treated for 3 to 17 months.[42] The initial median threshold milk dose (range) was 500 mg (500-4,000 mg). Fourteen children were able to significantly escalate daily doses by a median 9-fold (range, 2- to 32-fold), with a maximum median tolerated daily dose of 7 g (range, 1-16 g). Follow-up milk challenges were timed according to the success of home dosing and were conducted within 13 to 75 weeks of open-label dosing. Six children tolerated 16 g, and 7 reacted at 3 to 16 g. Adverse reactions were common and unpredictable, with several systemic reactions occurring at previously tolerated doses, often in association with exercise or febrile illness. The overall rate of reactions decreased over time, although 1 child had symptoms suggesting possible eosinophilic gastrointestinal disease.

Longo et al[43] evaluated the safety and efficacy of OIT in 60 children with severe cow's milk allergy. Subjects enrolled had milk-specific IgE levels of greater than 85 kU_A/L and reacted to 0.8 mL or less of milk during an initial oral milk challenge. Thirty children were randomized to OIT with a 10-day rush phase in the hospital and a slow dose-escalation phase at home (increasing by 1 mL every other day). Thirty children were randomized to an untreated comparison group. After 1 year, 11 (36%) of 30 children in the OIT group were able to ingest a daily dose of milk equal or greater than 150 mL, whereas 16 (54%) children were able to ingest from 5 mL to less than 150 mL. Three (10%) children were unable to complete the OIT because of the ongoing adverse reactions. All 30 children in the comparison group reacted to less than 5 mL of milk during the follow-up challenge. Adverse reactions, including systemic reactions, were common in both groups, but no child had severe anaphylaxis. Intramuscular epinephrine was administered 4 times in 4 children during the rush phase and twice in 1 child during the home phase. In addition, 24 children received nebulized epinephrine: 18 were treated with 22 doses of inhaled epinephrine during the rush phase, and 6 were treated with 9 doses during the home phase.

Peanut OIT

Peanut OIT trials in young children with peanut allergy have attracted significant attention (Table II).[44-46] In one study 39 children (median age, 57.5 months; range, 12-111 months; 64% male) were enrolled in an open-label uncontrolled trial of peanut OIT.[44] Pretherapy oral food challenges were not performed. All children completed the initial-day escalation phase up to 50 mg, although 36 experienced adverse symptoms. During the build-up phase, children ingested peanut flour with vehicle daily; doses were increased by 25 mg every 2 weeks until 300 mg was reached. After 4 to 22 months of maintenance therapy, an oral food challenge was performed, and 27 of 29 children tolerated 3.9 g of peanut. Children were evaluated every 4 months during continued maintenance dosing, for a total of 36 months. Ten (25%) children withdrew after the initial-day escalation phase. Six withdrew for personal reasons, and 4 withdrew because of allergic reactions to the OIT that did not resolve with continued treatment or dose reduction. Three had gastrointestinal complaints, and 1 had asthma. Twenty-nine subjects completed all 3 phases of the study and peanut challenges.

During the initial escalation phase, 36 (92%) patients experienced adverse symptoms; most common (27 [69%] patients) included upper respiratory tract symptoms, such as mild sneezing, itching, and mild laryngeal symptoms. A total of 6 (15%) patients had mild wheezing, and 2 of them progressed to moderate wheezing. During the build-up phase, adverse symptoms occurred after 46% of the doses. The risk of an adverse reaction with any home dose was 3.5% (upper respiratory tract, 1.2%; skin, 1.1%). Treatment was administered for reactions after 0.7% of home doses, including 1 intramuscular epinephrine injection in 2 subjects. By 6 months, titrated skin prick test responses and activation of basophils decreased significantly. Peanut-specific IgE antibody concentrations decreased by 12 to 18 months, whereas peanut-specific IgG4 antibody concentrations increased significantly. Serum factors inhibited IgE-peanut complex formation in an IgE-facilitated allergen-binding assay, and secretion of IL-10, IL-5, IFN-γ, and TNF-α from peanut-stimulated PBMCs *in vitro* increased over a period of 6 to 12 months. Peanut-specific $FoxP3^+$ regulatory T-cell numbers increased until 12 months and decreased thereafter, and T-cell microarrays showed downregulation of genes involved in the apoptotic pathways.

In a German study 23 children (median age, 5.6 years; range, 3.2-14.3 years) with severe peanut allergy confirmed by means of DBPCFC received OIT with roasted peanut.[46] The median peanut-specific IgE level was 95.6 kU_A/L (range, 3-2,071 kU_A/L). After the baseline DBPCFC, rush OIT was initiated in the hospital with increasing doses of crushed roasted peanuts 2 to 4 times per day for up to 7 days. The starting dose was equal to approximately 1% of the threshold dose during the baseline peanut challenge. If a protective dose of at least 500 mg of peanut was not achieved, children continued with a long-term build-up protocol using biweekly dose increases up to the maintenance dose of at least 500 mg. After 8 weeks of maintenance therapy, therapy was discontinued for 2 weeks before conducting the final DBPCFC. After a median of 7 months, 14 (60%) of 23 children reached the protective dose of 500 mg of peanut. Overall, 2.6% of 6,137 OIT doses provoked adverse symptoms, and lower respiratory tract symptoms were observed in 1.3% of doses. At the final DBPCFC, children tolerated a median of 1,000 mg (range, 250-4,000 mg) compared with a median 190 mg (range, 20-1,000 mg) of peanut during the baseline DBPCFC. There was a significant increase in peanut-specific serum IgG4 and a decrease in peanut-induced IL-5, IL-4, and IL-2 production by PBMCs *in vitro* after OIT.

Patterns of response to food OIT

Distinct patterns of response to OIT emerge from the published studies (Fig 2).[38,41,44,46,50] Approximately 10% to 20% of patients fail the initial rush/escalation phase (desensitization failure) and withdraw from the protocols because of significant adverse reactions; 10% to 20% do not achieve the full planned maintenance dose (partial desensitization). Overall, approximately 50% to 75% achieve and tolerate the maintenance dose. The majority of children tolerate more than 5 g of the allergenic food during therapy, but it remains to be determined whether partially desensitized subjects might become tolerant with a longer duration of OIT. It is also unclear whether failure of desensitization is associated with the most severe and likely permanent food

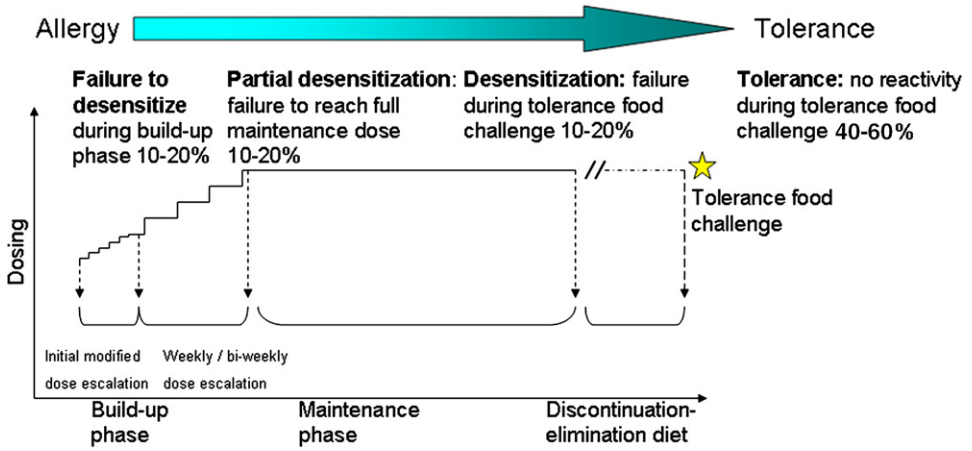

FIG 2. Patterns of response to the food OIT.

allergy phenotype, as opposed to the successful desensitization and tolerance induction that might be associated with a transient clinical phenotype and higher chances of spontaneous resolution of food allergy.

SLIT

SLIT with food allergens represents another approach to desensitization and possible tolerance. The first report described SLIT with fresh kiwi pulp extract in a 29-year-old woman with a history of kiwi-induced anaphylaxis.[53] A randomized, double-blind, placebo-controlled trial of SLIT for hazelnut allergy was conducted in adults with hazelnut allergy (54.5% with a history of oral allergy symptoms) confirmed by means of DBPCFC.[54,55] Subjects were randomly assigned to one of 2 groups: hazelnut immunotherapy (n = 12) or placebo (n = 11). Treatment extract solution was held in the mouth for at least 3 minutes and then spit out. All subjects receiving hazelnut SLIT reached the planned maximum dose with a 4-day rush protocol, followed by a daily maintenance dose (containing 188.2 μg of Cor a 1 and 121.9 μg of Cor a 8, major hazelnut allergens). Systemic reactions were observed in 0.2% of the total doses during the rush build-up phase and were treated with oral antihistamines. Local reactions, mainly immediate oral pruritus, were observed in 7.4% of doses (109 reactions/1,466 doses). Four patients in the active SLIT group reported abdominal pain several hours after dosing during the build-up phase. Local reactions during the maintenance phase were limited to oral pruritus and occurred in 1 patient. After 5 months of SLIT, the mean threshold dose of ingested hazelnut increased from 2.3 to 11.6 g in the active group ($P = .02$) compared with 3.5 to 4.1 g in the placebo group (nonsignificant). Almost 50% of treated subjects tolerated the highest dose (20 g) of hazelnut during follow-up DBPCFCs compared with 9% of the placebo group. Levels of serum hazelnut-specific IgG4 antibody and total serum IL-10 increased only in the active group, but there were no differences in hazelnut-specific IgE antibody levels before and after immunotherapy.

In a double-blind, placebo-controlled study, 18 children 1 to 11 years of age were randomized 1:1 to peanut SLIT or placebo administered as 6 months of dose escalation and 6 months of maintenance followed by a peanut DBPCFC.[56] Crude peanut extract (1:20 wt/vol in 0.2% phenol and 50% glycerinated saline) contained the maximum peanut protein concentration of 500 μg/mL; the placebo was a solution of glycerinated saline with phenol and caramel coloring. The study drug was administered sublingually, held for 2 minutes, and then swallowed. Although DBPCFCs were not performed before therapy, the active treatment group reacted at a threshold dose 20 times higher than that for the placebo group (median 1,710 vs 85 mg; $P = .01$) during the DBPCFC after 12 months of therapy. The active treatment group demonstrated significantly decreased skin prick test wheal size, decreased basophil responsiveness to peanut stimulation, and increased peanut IgG4 and decreased IL-5 levels after 12 months. Peanut IgE levels increased over the first 4 months and then steadily decreased. Side effects were primarily local oropharyngeal symptoms and were observed with 11.5% of active and 8.6% of placebo doses. Of the 4,182 active doses, 11 (0.3%) required treatment with an oral antihistamine; 1 episode of mild wheezing was treated with nebulized albuterol. Twelve months of peanut SLIT induced clinical desensitization. Long-term study is required to assess tolerance.

An uncontrolled pilot study of SLIT was done in 8 children with cow's milk allergy.[57] After an initial positive oral milk challenge, children started SLIT with 0.1 mL of milk for the first 2 weeks, increasing by 0.1 mL every 15 days until 1 mL/d was taken. Milk was kept in the mouth for 2 minutes and then spit out. Seven children completed the protocol, and 1 child withdrew because of continued oral symptoms. After 6 months of treatment, the threshold dose of milk increased from a mean of 39 mL at baseline to 143 mL ($P < .01$).

A randomized, double-blind, placebo-controlled trial of SLIT with Pru p 3 (major peach allergen) in adults with peach allergy found a beneficial effect of SLIT at a maintenance dose of 50 μg of Pru p 3 for 6 months.[58] In the SLIT-treated subjects (n = 37) the threshold doses of Pru p 3 for local reactions (usually oral pruritus) during a DBPCFC were 9 times higher and for systemic reactions (usually transient gastrointestinal discomfort or mild rhinitis) were 3 times higher after 6 months of SLIT compared with pre-SLIT threshold doses. In contrast, the placebo-treated subjects experienced no significant changes in their eliciting doses of Pru p 3. Specific IgE levels to recombinant Pru p 3 increased both in the active ($P < .001$) and placebo ($P = .03$) groups, although the increase remained only significant at 6 months in the active group (active group, 4.2 [$P < .001$]; placebo group, 4.0 [$P = .08$]; *t* test). IgG4 levels to native Pru p 3 increased significantly in the active group ($P = .007$) but not in the placebo group ($P = .2$). Peach SLIT was reportedly well tolerated.

Preliminary data on food OIT and SLIT suggest a beneficial treatment effect, although significant adverse reactions in the former are common. Before these treatments can be used in clinical practice, additional studies are needed to determine optimal maintenance doses, ideal duration, degree of protection, efficacy for different ages, severity and type of food allergies responsive to treatment, and the need for patient protection during home administration.[59,60]

EPIT

An alternative route of allergen delivery is through an epicutaneous patch. In a small pilot study 18 children (mean age, 3.8 years; range, 10 months to 7.7 years) with cow's milk allergy were randomized 1:1 to receive active EPIT or placebo.[61] Cow's milk allergy was confirmed by a clinician-supervised oral food challenge at baseline, and the threshold dose of milk was established. Children received three 48-hour applications (1 mg of skimmed milk powder or 1 mg of glucose as placebo) through the skin patch per week for 3 months. EPIT-treated children had a trend toward increased threshold doses at the follow-up oral milk challenge, from a mean of 1.8 mL at baseline to 23.6 mL at 3 months; there was no change in the placebo group. There were no significant changes in cow's milk–specific IgE levels from baseline to 3 months in either group. The most common side effects were local pruritus and eczema at the site of EPIT application. There were no severe systemic reactions; however, 1 child had repeated episodes of diarrhea after EPIT. This small pilot study suggests that further investigation of EPIT for food allergy is warranted.

IMMUNOTHERAPY WITH MODIFIED RECOMBINANT ENGINEERED FOOD PROTEINS

The risk of an immediate allergic reaction during immunotherapy can be decreased by modifying the IgE antibody–binding sites (epitopes) with point mutations introduced by site-directed mutagenesis or with protein polymerization (Table III).[62-70] Modified food allergens can be combined with bacterial adjuvants (eg, heat-killed *Listeria moncytogenes* [HKLM] or HKE) to enhance the T_H1-skewing effect and decrease the T_H2-skewing effect. C3H/HeJ mice with peanut allergy were treated subcutaneously 10 weeks after sensitization with a mixture of the recombinant modified major peanut allergens and HKLM (mAra h 1-3 plus HKLM).[71] All mice in the sham-treated group had anaphylactic symptoms, whereas only 31% of the mice in the mAra h 1-3 plus HKLM group had mild anaphylaxis during a posttreatment oral peanut challenge. In subsequent studies a nonpathogenic strain of *E coli* containing the modified peanut proteins was used as an adjuvant, and the vaccine was administered through the oral, nasal, subcutaneous, and rectal routes. Oral delivery was not effective, presumably because of breakdown of the peanut-containing *E coli*. Although the nasal and subcutaneous routes were effective, rectal delivery was selected for further study because of safety concerns because nonpathogenic *E coli* bacteria reside in the colon. C3H/HeJ mice with peanut allergy received 0.9 μg (low dose), 9 μg (medium dose), or 90 μg (high dose of HKE expressing the modified Ara h 1-3 proteins [HKE-MP123]) per rectum, HKE-containing vector (HKE-V) alone, or vehicle alone (sham) weekly for 3 weeks.[63] Mice were challenged with peanut 2 weeks after the final vaccine dose and then at monthly intervals for 2 more months. After the first peanut challenge, the groups receiving all 3 doses of HKE-MP123 and the HKE-V–treated group had reduced severity of anaphylaxis ($P < .01$, .01, .05, and .05, respectively) compared with that seen in the sham-treated group. However, only the medium- and high-dose HKE-MP123–treated mice remained protected for up to 10 weeks after treatment. Peanut-specific IgE levels were significantly less in all HKE-MP123–treated groups ($P < .001$) but were most reduced in the high-dose HKE-MP123–treated group at the time of each challenge. *In vitro* peanut-stimulated splenocytes from the high-dose HKE-MP123–treated mice produced significantly less IL-4, IL-13, IL-5, and IL-10 ($P < .01$, .001, .001, and .001, respectively). IFN-γ and TGF-β synthesis were significantly increased ($P < .00001$ and .01, respectively) compared with that seen in sham-treated mice at the time of the last challenge. A phase I clinical safety study is currently enrolling adult subjects with peanut allergy. In future studies, probiotic bacteria might also be used as bacterial adjuvants to avoid the concerns of excessive T_H1 stimulation by killed pathogenic bacteria.[72]

Other approaches

Several additional approaches to peanut allergy have been evaluated in animal studies, as outlined in Table III. In peptide immunotherapy the antigen-presenting cells are provided with T-cell epitopes in the absence of a second signal, and mast cells are not activated because the short peptides are unable to cross-link 2 IgE molecules.[64,73] Immunization with bacterial plasmid DNA (pDNA) that encodes specific antigens can induce prolonged humoral and cellular immune T_H1 responses. The T_H1 effect is mediated by immunostimulatory sequences (ISSs) consisting of unmethylated cytosine and guanine motifs (CpG motifs) in the bacterial pDNA backbone. Intramuscular immunization of naive AKR/J ($H\text{-}2^K$) and C3H/HeJ ($H\text{-}2^K$) mice with pDNA encoding Ara h 2 before intraperitoneal peanut sensitization had a protective effect in AKR/J mice but induced anaphylactic reactions in C3H/HeJ mice after peanut challenge.[65] In another study oral chitosan-embedded Ara h 2 had a protective effect in AKR mice.[74] These studies raise concerns that the effect of pDNA-based immunotherapy might be strain dependent and not universally effective in reversing IgE-mediated food hypersensitivity in human subjects.

Synthetic immunostimulatory oligodeoxynucleotides containing unmethylated CpG motifs (ISSs) linked to allergenic proteins represent an alternative approach to DNA-based immunotherapy. ISS-linked Ara h 2 administration was effective in the suppression of anaphylactic symptoms compared with that seen in sham controls.[66] Similarly, intradermal immunization with a mixture of ISSs and β-galactosidase provided protection against fatal anaphylaxis induced by intraperitoneal β-galactosidase sensitization and challenge that was comparable with protection provided by immunization with the pDNA encoding β-galactosidase.[75] Protection was associated with an increase in IgG2a/IFN-γ levels and a decrease in IgE, IL-4, and IL-5 levels. ISS-linked allergen immunization might have a prophylactic effect against food allergy; however, the ability to reverse established food allergy remains to be determined.

Other novel therapeutic approaches that might be used to treat food allergy include human immunoglobulin Fc-Fc fusion proteins that cross-link the high-affinity FcεRI and low-affinity FcγRIIb receptors on mast cells and basophils, leading to inhibition of degranulation.[67-69] Because many major food allergens have been identified, this approach might be applied to food allergy therapy. C-type lectin receptors on dendritic cells, such as SIGNR-1 (also

TABLE III. Modified recombinant allergen immunotherapy for food allergy

Therapy	Mechanism of action	Effects	Comments
Clinical			
Heat-killed bacteria mixed with or containing modified peanut proteins Li et al, 2003[63]	Upregulation of T_H1 and regulatory T-cell cytokine responses	Protection against peanut-induced anaphylaxis in mice, lasting up to 10 wk after treatment	Concern for toxicity of bacterial adjuvants, excessive T_H1 stimulation, and potential for autoimmunity. Heat-killed *E coli* expressing modified peanut allergens administered rectally is viewed as the safest approach for future human studies. A phase I clinical trial in adults with peanut allergy is ongoing.
Preclinical (murine models)			
Peptide immunotherapy Li et al, 2001[64]	Overlapping peptides (10-20 amino acids long) that represent the entire sequence of allergen. Binding to mast cells is eliminated, and T-cell responses are preserved.	Protection against peanut-induced anaphylaxis in mice	Improved safety profile compared with conventional immunotherapy; does not require identification of IgE-binding epitopes
pDNA immunotherapy Li et al, 1999[65]	Induces prolonged humoral and cellular responses caused by CpG motifs in the DNA backbone	Protection against peanut-induced anaphylaxis in sensitized AKR/J mice but induction of anaphylaxis in C3H/HeJ ($H\text{-}2^K$) mice; no effect on peanut-specific IgE antibody levels	Serious concerns regarding safety in view of strain-dependent effects in mice and concern for excessive T_H1 stimulation and autoimmunity
ISS immunotherapy (ISS-ODN) Srivastava et al, 2001[66]	Potent stimulation of T_H1 through activation of antigen-presenting cells, natural killer cells, and B cells; increased T_H1 cytokine levels	Protection against peanut sensitization in mice	Not shown to reverse established peanut allergy, concern for excessive T_H1 stimulation, and potential for autoimmunity
Engineered recombinant peanut immunotherapy Srivastava et al, 2002[62]	Binding to mast cells eliminated or markedly decreased, T-cell responses comparable with those to native peanut allergens	Protection against peanut-induced anaphylaxis in mice	Improved safety profile compared with conventional immunotherapy, requires identification of IgE-binding sites
Human immunoglobulin Fc-Fc fusion protein Zhang et al, 2004[67] Kepley et al, 2004[68] Zhu et al, 2005[69]	Fusion protein cross-links the high-affinity FcεRI and low-affinity FCγRIIb receptors on mast cells and basophils.	Fusion protein inhibits degranulation of mast cells and basophils.	A human γ-allergen fusion protein, the Fc–Fel d 1 fusion protein, inhibited Fel d 1–mediated degranulation in purified human basophils from patients with cat allergy and blocked the allergic responses in a murine model. A similar approach can be used for food allergy.
Sugar-conjugated BSA Zhou et al, 2010[70]	Mannoside-conjugated BSA (Man_{51}-BSA) targeted lamina propria dendritic cells expressing SIGNR-1 and promoted $CD4^+$ type 1 regulatory T cells.	Mice sensitized with Man_{51}-BSA were protected from anaphylaxis during an oral challenge with BSA and Man_{51}-BSA.	Sugar-modified food allergens might be used to induce oral tolerance by targeting SIGNR-1 and lamina propria dendritic cells.

called CD209b), might play a role in promoting oral tolerance development and thus preventing food-induced anaphylaxis.[70] Mice sensitized with mannoside-conjugated BSA (Man_{51}-BSA) were protected from anaphylaxis during an oral challenge with BSA and Man_{51}-BSA, whereas mice sensitized with BSA alone had significant allergic symptoms during oral challenge with BSA. Man_{51}-BSA selectively targeted lamina propria dendritic cells that expressed SIGNR-1 and induced the expression of IL-10, but not IL-6 or IL-12p70, promoting the generation of $CD4^+$ type 1 regulatory T cells. These findings suggest that sugar-modified food antigens might be used to induce oral tolerance by targeting SIGNR-1 and lamina propria dendritic cells.

ALLERGEN-NONSPECIFIC THERAPY

Humanized monoclonal anti-IgE

Humanized monoclonal murine anti-IgE IgG1 antibodies have been produced that bind to the constant region (third domain of the Fc region) of IgE antibody molecules and prevent IgE from binding to high-affinity FcεRI receptors expressed on the surface of mast cells and basophils and low-affinity FcεRII receptors expressed on B cells, dendritic cells, and intestinal epithelial cells. With the decrease in free IgE molecules caused by anti-IgE therapy, the expression of FcεRI receptors on mast cells and basophils is downregulated, resulting in decreased activation and release of histamine and other inflammatory mediators.[76] In

TABLE IV. Allergen-nonspecific therapy for food allergy

Therapy	Mechanism of action	Effects	Comments
Clinical trials			
Monoclonal anti-IgE Leung et al, 2003[77]	Binds to circulating IgE and prevents IgE deposition on mast cells and blocks degranulation; interferes with the IgE-facilitated antigen presentation by B cells and dendritic cells	Improves symptoms of asthma and allergic rhinitis and provides protection against peanut-induced anaphylaxis in 75% of treated patients (highest-dose group)	Subcutaneous at monthly or 2-wk intervals, unknown long-term consequences of IgE elimination; food nonspecific; can be used in combination with specific food allergen OIT
Chinese herbs FAHF-2 Wang et al, 2009[11] Wang et al, 2010[78]	Upregulation of T_H1 cytokines: IFN-γ, IL-12 Downregulation of T_H2 cytokines: IL-4, IL-5, IL-13; decreased allergen-specific IgE levels and T-cell proliferation to peanut	Reverses allergic inflammation in the airways, protects mice from peanut-induced anaphylaxis for prolonged periods of time	Oral, generally safe and well tolerated. Current studies focus on identification of the crucial active herbal components in the 9-herb formula and establishing optimal dosing in human phase I and II trials.
Monoclonal anti–IL-5 antibody (mepolizumab) Straumann et al, 2010[82]	Reduced tenascin C ($P = 0.03$) and TGF-β1 ($P = .05$) expression in the esophageal epithelial layer 13 wk after initiation of treatment	Limited improvement of symptoms: a trend was seen between 4 and 13 wk after initiation of mepolizumab treatment.	Well tolerated; acceptable safety profile, even at the high 1,500-mg dose level. Current studies evaluate mepolizumab in children with EoE.
Trichuris suis ova therapy Summers et al, 2005[80,81]	Stimulation of IL-10 synthesis	In a murine model of food allergy protection against food-specific IgE sensitization and anaphylaxis	Safe and afforded clinical improvement in Crohn disease and ulcerative colitis; no effect in adults with allergic rhinitis
Preclinical (murine models)			
Lactococcus lactis transfected with IL-10 Frossard et al, 2007[72]	Decreased serum IgE and IgG1 levels; increased gut IgA and increased gut and serum IL-10 levels	Pretreatment of young mice before sensitization with β-lactoglobulin in the presence of cholera toxin protected against anaphylaxis on the oral food challenge.	This approach was only tested in the murine model; however, the concept of probiotic bacteria can be applied to delivery of engineered allergens in human studies.
Lactococcus lactis transfected with IL-12 and β-lactoglobulin Cortes-Perez et al, 2009[83]	Decreased IgG1 levels in serum and bronchoalveolar lavage fluid; decreased IL-4 and increased IFN-γ production by β-lactoglobulin stimulated splenocytes	Intranasal coadministration of live *L lactis* transfected with IL-12 and β-lactoglobulin inhibited allergic reactions in mice.	Probiotic bacteria engineered to deliver IL-12 and food allergen might be useful for preventing IgE sensitization to food allergens.
TLR9 agonist Zhu et al, 2007[79]	Induction of mucosal and systemic T_H1 responses; decreased peanut-specific IgE and IgG2 levels	Oral administration of TLR9 agonists decreased gastrointestinal inflammation and protected mice from peanut-induced anaphylaxis.	Protective effect was observed when TLR9 agonist was administered during sensitization, as well as in already sensitized mice.

addition, anti-IgE inhibits IgE-facilitated antigen uptake by B cells and antigen-presenting cells and might inhibit IgE antibody synthesis.

A multicenter clinical trial assessed the effect of humanized monoclonal anti-IgE murine IgG1 antibody (Hu-901) in 84 adults with peanut allergy (Table IV).[11,72,77-83] Peanut allergy was confirmed by means of peanut double-blind, placebo-controlled oral challenges, and the threshold dose of peanut protein eliciting objective symptoms was established. Subjects were randomized 3:1 to receive either the humanized mAb Hu-901 at 3 different doses (150, 300, or 450 mg) or placebo subcutaneously monthly for 4 doses. Oral peanut challenges were repeated within 2 to 4 weeks after the fourth dose of anti-IgE. The eliciting threshold dose showed an increasing trend over baseline in all 3 groups with an apparent dose response, but the increase was statistically significant only in the group treated with the highest anti-IgE dose (450 mg). In this group the threshold dose increased from approximately one half of a peanut kernel (178 mg) to almost 9 peanut kernels (2,805 mg; $P < .001$ for the comparison of the 450-mg dose with placebo and $P < .001$ for trend with increasing dose). However, approximately 25% of subjects treated with the highest dose of Hu-901 showed no change in their threshold dose, suggesting that a subset of patients might not benefit from the anti-IgE therapy or might require higher doses for protection.

A controlled trial of a different anti-IgE humanized IgG1 antibody molecule (omalizumab [Xolair]) in patients with peanut allergy was terminated because of the occurrence of 2 severe allergic reactions during the initial screening peanut challenge that raised safety concerns. Before discontinuing the trial, 26 subjects had been randomized 2:1 to Xolair or placebo and completed 24 weeks of therapy followed by a second DBPCFC.[84] Subjects in the Xolair arm appeared to experience a greater shift in tolerability than the placebo-treated group ($P = .054$).

The combination of anti-IgE and specific allergen immunotherapy has been investigated with environmental aeroallergens but not yet with food allergens.[85] The combination of anti-IgE and

food OIT has the hypothetical advantage of decreasing the risk of adverse reactions associated with OIT and decreasing facilitated antigen presentation, which promotes T_H2 responses. A study of anti-IgE and milk OIT in children and adults with milk allergy is currently ongoing.

Traditional Chinese medicine

Herbs have been used in traditional Chinese medicine (TCM) for many centuries, although not for food allergies. The initial study of TCM in food allergy used an herbal formula (Food Allergy Herbal Formula [FAHF] 1) containing a mixture of 11 herbs in a murine model of peanut-induced anaphylaxis.[86] Herbs included in FAHF-1 have been used for treating parasitic infections, gastroenteritis, and asthma by practitioners of TCM. FAHF-1 protected mice with peanut allergy against peanut-induced anaphylaxis. It reduced mast cell degranulation and histamine release, decreased peanut-specific serum IgE levels, and reduced peanut-induced *in vitro* lymphocyte proliferation, as well as the synthesis of IL-4, IL-5, and IL-13 but not IFN-γ. FAHF-1 had no observable toxic effects on the liver or kidneys, even at the highest doses.

A simplified formula, FAHF-2, composed of 9 herbs completely blocked anaphylaxis during peanut challenge up to 5 months after therapy.[87] This protective effect was mediated by IFN-γ produced by $CD8^+$ T cells.[88,89] Each herb provided some degree of protection from peanut-induced anaphylaxis, but none of them offered protection that was equivalent to that seen with the complete FAHF-2 mixture of herbs, suggesting synergy among the different ingredients.

A phase I, randomized, double-blind, placebo-controlled, dose-escalation study in 19 subjects (12-45 years) with peanut and tree nut allergy recently reported that FAHF-2 was safe and well tolerated.[78] Two patients (1 in the FAHF-2 group and 1 in the placebo group) reported mild gastrointestinal symptoms. Serum IL-5 levels decreased in the active treatment group after 7 days of treatment with FAHF-2. *In vitro* supernatant levels of IL-5 decreased, whereas IFN-γ and IL-10 levels increased in allergen-stimulated PBMCs cultured with FAHF-2. A phase II extended safety and efficacy trial is currently enrolling subjects 12 to 45 years of age with peanut, tree nut, sesame, fish, or shellfish allergy.

Probiotics

Probiotics are live bacteria or their components that have beneficial effects on the health of the host, presumably by improving intestinal microbial balance. The major sources of probiotics are dairy products that contain *Lactobacillus* and *Bifidobacterium* species. Potential mechanisms of probiotic immunomodulation include increased synthesis of IgA and IL-10, suppression of TNF-α, inhibition of casein-induced T-cell activation and circulating soluble CD4, and Toll-like receptor 4 signaling.[90]

In a murine model of shrimp-induced anaphylaxis, oral administration of a mixture of probiotics significantly reduced symptom scores and histamine release in the feces after shrimp tropomyosin oral challenge and serum shrimp-specific IgE levels. In the jejunum IL-4, IL-5, and IL-13 tissue content was significantly reduced, whereas *FOXP3* and *IL27* mRNA expression and IL-10, TGF-β, and IFN-γ tissue content were upregulated.[91]

Clinical trials of probiotics have focused on the prevention and treatment of atopic dermatitis, which includes a large subset of children with food allergy. It has been hypothesized that the defective skin barrier resulting from atopic inflammation predisposes infants to IgE-mediated responses to food and environmental allergens.[92] Therefore it has been suggested that strategies to improve skin barrier function would decrease the risk of food sensitization. Prenatal supplementation of mothers and postnatal supplementation of infants during the first 6 months of life have been reported to decrease the prevalence of atopic dermatitis at 2 and 7 years of age, without any effect on IgE sensitization to food or environmental allergens.[93] Other studies have not replicated this finding.[94,95]

Prebiotics are oligosaccharides that promote probiotic colonization of the gastrointestinal tract. In a large clinical trial of 830 healthy term infants at low risk for atopy,[96] the cumulative prevalence of atopic dermatitis at 1 year of age was reportedly 5.7% infants in the prebiotic group compared with 9.7% infants in the control group ($P = .04$). However a double-blind, randomized, placebo-controlled trial in 119 infants with cow's milk allergy treated with a mix of 2 probiotics for 12 months showed no benefit for cow's milk allergy.[97] There was no difference in the cumulative percentage of tolerance to cow's milk at 6 and 12 months: 56 (77%) in the probiotic group versus 54 (81%) in the placebo group.

Lactococcus lactis–expressing IL-10 and IL-12

L lactis transfected to secrete murine IL-10 (*L lactis*-IL-10) was administered to young mice before oral sensitization with β-lactoglobulin and cholera toxin.[72] Pretreatment with *L lactis*-IL-10 diminished anaphylaxis severity and inhibited serum β-lactoglobulin IgE and IgG1 production and increased β-lactoglobulin IgA production in the gut. *L lactis*-IL-10 induced IL-10 secretion by Peyers patch cells in the gut and increased plasma IL-10 titers.

Intranasal coadministration of live *L lactis* transfected with IL-12 and β-lactoglobulin inhibited allergic reactions in mice. Treatment with *L lactis*–IL-12–β-lactoglobulin, but not with β-lactoglobulin alone, decreased IgG1 production in serum and bronchoalveolar lavage fluid. There was also decreased IL-4 production and enhanced IFN-γ production by β-lactoglobulin–stimulated splenocytes, indicating a switch from a T_H2 to T_H1 immune response.[98]

These results suggest that probiotic bacteria engineered to deliver IL-10 or IL-12 might be able to decrease food-induced anaphylaxis and provide a treatment option to prevent IgE-type sensitization to food allergens.

Toll-like receptors

Signaling through Toll-like receptor 9 (TLR9) induces mucosal and systemic T_H1 immune responses. Oral administration of a synthetic TLR9 agonist resulted in decreased gastrointestinal inflammation and protection from peanut-induced anaphylaxis in a murine model of peanut allergy.[79] The protective effect included decreased levels of peanut-specific IgE and IgG2 antibodies; protection was observed when TLR9 agonist was administered both during and after sensitization to peanut.

Trichuris suis ova therapy

Parasitic helminth infections can protect against allergic airway inflammation in experimental models and have been associated with a reduced risk of atopy and a milder course of asthma in some observational studies.[99-101] In a murine model of food allergy, helminth infection was reportedly protective against

IgE sensitization and anaphylaxis by stimulating IL-10.[102,103] The helminth *T suis* has been shown to be safe and beneficial in clinical trials of ulcerative colitis and Crohn disease.[80,81] Although the approach of controlled helminthic infection is controversial, *T suis* ova therapy's efficacy in patients with inflammatory bowel disease and the data from murine models of food allergy provide a logical rationale for extending the investigation into food allergy. However, a recent study of *T suis* ova therapy in adults with allergic rhinitis found no beneficial effect on symptoms scores, days without symptoms, total histamine levels, grass-specific IgE levels, or diameters of wheal reactions on skin prick testing with grass pollen.[104]

Anti–IL-5 antibody (mepolizumab) in patients with eosinophilic esophagitis

Eosinophilic esophagitis (EoE) is a disorder of mixed pathophysiology, with both IgE-mediated and non–IgE-mediated mechanisms involved. A subset of subjects with EoE is responsive to food elimination, especially in children. Considering the pivotal role of IL-5 in the accumulation of eosinophils in the esophageal tissue, treatment with an anti–IL-5 mAb was investigated in a randomized, placebo-controlled, double-blind trial.[82] Adults with active EoE were randomized to receive 750 mg of mepolizumab (n = 5) or placebo (n = 6). A significant reduction of mean esophageal eosinophilia was seen in the mepolizumab-treated group (−54%) compared with the placebo group (−5%) after the first dose ($P = .03$), but limited improvement of clinical symptoms was observed. Mepolizumab was well tolerated and had an acceptable safety profile. Currently, mepolizumab is being evaluated in children with EoE.

"An ounce of prevention is worth a pound of cure" (Benjamin Franklin, 1706-1790)

A reassessment of neonatal feeding studies prompted the European and American pediatric societies to alter previous feeding guidelines for mothers and newborns. In recognition of an apparent lack of effect of intrauterine and early-life avoidance of peanut feeding, the guidelines no longer stress allergen avoidance by mothers during pregnancy or while breast feeding or by their newborns.[105] In fact, 3 large cohort studies have provided compelling evidence that early introduction of peanut, milk, and egg into an infant's diet might decrease the risk of IgE-mediated allergy to those foods.[106-108] In addition, epidemiologic observations and studies in animal models have highlighted the potential for sensitization to peanut and egg white through cutaneous contact. This route favors a T_H2-skewed immune response and specific IgE production, which suggests a need for early oral introduction to counter the effect of cutaneous exposure.[109-112] However, in a recent study of infants at high risk of peanut allergy, a direct correlation was found between the degree of sensitization in infants and the amount of peanut consumed by their mothers during the third trimester of pregnancy.[113] Several ongoing studies should help clarify these issues over the next several years.

CONCLUSIONS

Food allergy is an increasingly prevalent problem in westernized countries, and there is an unmet medical need for an effective therapy for this allergy. Among the plethora of novel approaches, the strategies most likely to advance into clinical practice include the Chinese herbal formula FAHF-2 and OIT alone or in combination with anti-IgE antibody. Diets containing extensively heated (baked) milk and egg represent an alternative approach to food OIT and are already changing the paradigm of strict dietary avoidance for patients with food allergy. The exponential increase in research activity on food allergy and the concerted efforts in major centers worldwide give hope that an effective treatment for food allergy is within reach.

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