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Future Therapies for Food Allergies

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Abstract

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, 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 non-specific. Allergen-specific approaches include oral, sublingual and epicutaneous immunotherapy (desensitization) with native food allergens, and mutated recombinant proteins, which have decreased IgE-binding activity, co-administered within heat-killed *E.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 food-allergic patients. Non-specific approaches include monoclonal anti-IgE antibodies, which may increase the threshold dose for food allergen in food-allergic patients, and a Chinese herbal formulation, which prevented peanut-induced anaphylaxis in a mouse 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.

Keywords

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 two decades food allergy has emerged as a major public health problem in westernized societies.^{1, 2} In American children under 18 years of age, 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, 5} Food allergy is the most common cause of anaphylaxis evaluated in the emergency departments 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 US and UK.^{3, 6, 7} Food-induced anaphylaxis occasionally results in fatalities, with over 90% of deaths in the US caused by reactions to peanut or tree nuts.^{8, 9}

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The current management of food allergy is limited to strict dietary avoidance, nutritional counseling, and emergency treatment of adverse reactions.¹⁰ In this review, we will focus on efforts to treat IgE-mediated forms of food allergy. Although attempts to desensitize food allergic patients date back more than 100 years, e.g. oral immunotherapy,¹¹ there are no accepted therapies proven 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, shellfish) or are most common in children, such as cow's milk and hen's egg.¹² Approaches being pursued are both food allergen-specific and non-specific.¹³ (Fig 1) Allergen-specific approaches include oral, sublingual and epicutaneous immunotherapy (desensitization) with native food allergens, and mutated recombinant proteins, which have decreased IgE-binding activity, co-administered within heat-killed *E.coli* to generate maximum immune response. Diets containing extensively heated (baked) food, e.g. milk or egg, may represent an alternative approach to allergen-specific immunomodulation of food allergy in some patients.

Non-specific approaches include monoclonal anti-IgE antibodies, which may increase the threshold dose for reactivity to food allergens, and a Chinese herbal formulation, which prevented peanut-induced anaphylaxis in a mouse model of peanut 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 food-allergic patients and their families. Fortunately about 85% of children allergic to foods such as cow milk, egg, wheat and other cereal grains, soy, etc. "outgrow" (develop tolerance) their allergy whereas only 15% - 20% of children allergic to peanut, tree nuts, fish and shellfish will develop tolerance spontaneously. Diagnostic tests are needed that can distinguish individuals with transient from persistent forms of food allergy so that therapeutic strategies can be employed 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 (e.g., serum food allergen-specific IgE antibodies or the skin prick test) that reliably predict the potential for spontaneous development of oral tolerance. However, two recent reports in children with multiple food allergies noted that few children with peak cow milk or egg white-specific IgE antibody levels ≥ 50 kU_A/L (UniCAP, Phadia) outgrow their allergy by their late teenage years.^{14, 15} In addition, recent studies utilizing peptide microarray assays to determine the diversity and affinity of IgE binding to sequential epitopes on major food allergens (e.g. peanut, cow milk and egg white) may be useful in determining the severity and persistence of food allergy in affected patients.¹⁶⁻²⁴ (Table I)

IMMUNOTHERAPEUTIC APPROACHES FOR TREATING FOOD ALLERGY

Food allergic patients 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 are the result of different immunologic mechanisms and therefore are 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 may present a more challenging situation. Patients with the persistent form of food allergy are likely to have a less favorable response to therapy, including failure to desensitize, failure to develop oral tolerance, need for a more prolonged

treatment course, and the development of more serious adverse reactions while on 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.^{16,17} Based on these observations, we hypothesized that children with transient milk and egg allergy, which comprises up to 80% of milk and egg allergic children, 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.^{25, 26} 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 prick skin tests 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 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).²⁷

Children who reacted to extensively heated milk had significantly higher basophil reactivity to stimulation with casein compared to the extensively heated milk-tolerant children.²⁸ There was a significantly higher median percentage (25th% to 75th%) 16.9% (7.1-31.7) of proliferating casein-specific CD25(+)CD27(+) T cells from casein-induced PBMC cultures of 18 extensively heated milk-tolerant subjects compared to 8 subjects reactive to extensively heated milk; 4.9% (2.6-7.5); $P < 0.01$.²⁹ There were no significant differences between the groups in the frequency of polyclonal T cells or casein-specific T effector cells. Casein-specific T regulatory cells were FoxP3(+)CD25(hi)CD27(+), CTLA 4(+), CD45RO(+)CD127(-). Depletion of the CD25(hi) cells before *in vitro* culture significantly enhanced casein-specific T effector cell expansion, confirming the presence of greater T regulatory activity. A higher frequency of casein-specific T regulatory 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 3-6 months. No increases were seen in acute allergic reactions or in 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 following the introduction of baked goods with milk and egg into the diet included increasing food-specific IgG₄ antibodies, decreasing wheal sizes from skin prick tests, and a trend for decreasing food-specific IgE antibodies; findings similar to those observed in oral immunotherapy. Preliminary findings suggest that many of the children placed 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 over 100 years to treat environmental allergies. In a study utilizing an aqueous extract of peanut, three actively treated subjects displayed a 67% to 100% decrease in symptoms during double-blind, placebo-controlled food challenges, and had a 2- to 5-log reduction in end-point skin prick test reactivity to peanut at the end of the treatment course, while one placebo-treated subject had no change in these parameters.³⁰ As a consequence of a pharmacy error, one placebo-treated subject died of anaphylaxis following 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 weight/volume peanut extract, and 6 were followed as an observational untreated control group for 12 months.³¹ 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 testing, whereas untreated controls experienced no improvement in those 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 due to frequent adverse reactions. During the maintenance phase, the rate of systemic reactions was 39%, with an average 12.6 epinephrine injections per subject. While 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 food in subjects treated with pollen subcutaneous or sublingual immunotherapy.^{32-35 36} An open trial of birch subcutaneous immunotherapy in 49 adults with birch allergic rhinitis and PFAS to apple found a significant reduction (50-95%) or complete resolution of apple-induced oral symptoms in 84% of treated subjects compared to no benefit in controls ($P < 0.001$) and a reduction in skin test reactivity to fresh apple in 88% subjects at the end of 12 month course of birch immunotherapy.³² In a follow-up study over 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 tests. 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.^{34, 37 35} Sublingual immunotherapy (SLIT) with birch pollen extract in adults with birch allergic rhinitis did not significantly reduce apple-induced PFAS symptoms.³⁸

The beneficial effects on PFAS were predominantly reported in adults mono-sensitized 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 may represent a superior approach to the treatment of PFAS.

FOOD ORAL IMMUNOTHERAPY

Successful oral immunotherapy (OIT) in a boy with egg-induced anaphylaxis was first reported in 1908.¹¹ At present, oral immunotherapy (OIT) to food is one of the most actively

investigated therapeutic approaches for food allergy, although few trials have established patient reactivity prior to therapy and/or included a placebo control. Furthermore, while studies suggest that a majority of food allergic patients can be desensitized with OIT, no studies have demonstrated the development of tolerance. In addition, adverse reactions during therapy are common, i.e. >25% of doses associated with adverse symptoms, although most are mild in nature. (Table II)

Oral tolerance versus desensitization

The ultimate goal of food allergy therapy is permanent oral tolerance, which is established when the food may 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 pro-allergic Th2 response, followed by anergy at later stages.³⁹ 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 may 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 may be tested with intentional interruption of dosing for at least 4 – 12 weeks followed by a supervised oral food challenge.^{40, 41}

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 occur at home. Early uncontrolled studies provided evidence that a subset of food allergic subjects could be “desensitized” to a variety of foods, including milk, egg, fish, fruit, peanut, and celery.⁴²⁻⁴⁵ Some subjects who tolerated a maintenance dose, even for a significant period of time, re-developed allergic symptoms if the food was not ingested on a regular basis, highlighting a concern that permanent tolerance was not achieved.⁴⁰

Milk OIT

In a large trial of OIT, 45 (median age 2.5 years, range 0.6–12.9 years) children with challenge-proven IgE-mediated cow milk or egg allergy were randomly assigned to OIT (n=25) or an elimination diet as a control group (n=20).⁴¹ OIT with fresh cow milk or lyophilized egg protein was given at home daily. Following a median 21 months, children in the OIT group were placed on an elimination diet for 2 months prior to a follow-up re-challenge to determine whether oral tolerance had been achieved. At the follow-up challenge, there was no difference in the rate of tolerance between the two groups: 9 of 25 (36%) OIT children showed permanent tolerance compared to 7 of 20 (35%) control children. Allergen-specific IgE decreased significantly in children who developed natural tolerance during the elimination diet ($P < 0.05$) as well as in those treated with OIT ($P < 0.001$). In addition, 3 children (12%) in the OIT group could tolerate milk and 4 children (16%) could tolerate increased amounts of milk/egg compared to baseline while on 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.⁴⁶ 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. Following OIT, the

median threshold dose in the active treatment group was 5,140 mg (range 2540-8140 mg), whereas all patients in the placebo group only tolerated 40 mg ($P = 0.0003$). All children in the active treatment group experienced adverse reactions due to 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 3-17 months.⁴⁷ The initial median threshold milk dose (range) was 500 mg (500 to 4000 mg). Fourteen children were able to significantly escalate daily doses by a median (range) 9-fold (2-to 32-fold), with a maximum median (range) tolerated daily dose of 7 gm (1 to 16 gm). 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 gm, and 7 reacted at 3 to 16 gm. 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 one child developed symptoms suggesting possible eosinophilic gastrointestinal disease.

Longo et al evaluated the safety and efficacy of OIT in 60 children with severe cow's milk allergy. Subjects enrolled had milk-specific IgE > 85 kU_A/L and reacted to ≤0.8 mL of milk during an initial oral milk challenge.⁴⁸ 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 <150 mL. Three children (10%) were unable to complete the OIT because of the ongoing adverse reactions. All 30 children in the comparison group reacted to less than 5mL 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.^{49, 50 51} (Table II) 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.⁴⁹ Pre-therapy 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. Following 4 – 22 months of maintenance therapy, an oral food challenge was performed and 27 of 29 children tolerated 3.9 gm of peanut. Children were evaluated every 4 months while on continued maintenance dosing; a total of 36 months. Ten (25%) children withdrew following 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 patients (92%) experienced adverse symptoms; most common (27 patients, 69%) included upper respiratory symptoms such as mild sneezing, itching and mild laryngeal symptoms. A total of six patients (15%) had mild wheezing; two

of them progressed to moderate wheezing. During the build-up phase, adverse symptoms occurred following 46% of the doses. The risk of an adverse reaction with any home dose was 3.5%; upper respiratory tract (1.2%) and skin (1.1%). Treatment was administered for reactions following 0.7% of home doses, including 1 intramuscular epinephrine injection in 2 subjects. By 6 months, titrated skin prick tests 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 peripheral blood mononuclear cells in vitro increased over a period of 6 to 12 months. Peanut-specific forkhead box protein-3 (Fox P3)-positive regulatory T cells increased until 12 months and decreased thereafter, and T-cell microarrays showed down-regulation of genes involved in the apoptotic pathways.

In a German study, 23 children [median (range) age 5.6 years (3.2-14.3)] with severe peanut allergy confirmed by DBPCFC received OIT with roasted peanut.⁵¹ The median (range) peanut-specific IgE was 95.6 kU_A/L (3-2071). Following the baseline DBPCFC, rush OIT was initiated in the hospital with increasing doses of crushed roasted peanuts, 2-4 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 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 of peanut. Following 8 weeks of maintenance therapy, therapy was discontinued for 2 weeks before conducting the final DBPCFC. After a median of 7 months, 14 of 23 (60%) children reached the protective dose of 500 mg of peanut. Overall, 2.6% of 6,137 OIT doses provoked adverse symptoms and lower respiratory symptoms were observed in 1.3% of doses. At the final DBPCFC, children tolerated a median of 1000 mg (range, 250-4000 mg) compared with a median 190 mg (range, 20-1000 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 following OIT.

Patterns of response to food oral immunotherapy

Distinct patterns of response to OIT emerge from the published studies.^{40, 41, 46, 49, 51} (Figure 2) Approximately 10-20 % of patients fail the initial rush / escalation phase (desensitization failure) and withdraw from the protocols due to significant adverse reactions; 10-20 % fail to achieve the full planned maintenance dose (partial desensitization). Overall, approximately 50-75% achieve and tolerate the maintenance dose. The majority of children tolerate > 5 gm of the allergenic food while on 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 allergy phenotype, as opposed to the successful desensitization and tolerance induction that may be associated with a transient clinical phenotype and higher chances of spontaneous resolution of food allergy.

Sublingual immunotherapy

Sublingual immunotherapy (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.⁵² 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 double-blind placebo-controlled food challenge.^{53, 54} 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/1466 doses). Four patients in the active SLIT group reported abdominal pain several hours following dosing during the build-up phase. Local reactions during the maintenance phase were limited to oral pruritus and occurred in one patient. After 5 months of SLIT, the mean threshold dose of ingested hazelnut increased from 2.3 g to 11.6 g in the active group ($P = 0.02$) compared to 3.5 g to 4.1 g in placebo (non-significant). Almost 50% of treated subjects tolerated the highest dose (20 g) of hazelnut during follow-up DBPCFCs, compared to 9% in 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 pre- and post-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 given as 6 months of dose escalation and 6 months of maintenance followed by a peanut DBPCFC.⁵⁵ Crude peanut extract 1:20 w/v in 0.2% phenol and 50% glycerinated saline contained maximum peanut protein concentration 500 mcg/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 prior to therapy, the active treatment group reacted at a threshold dose 20 times higher than the placebo group, median 1710 mg versus 85 mg, $P = 0.01$ during the DBPCFC following 12 months of therapy. The active treatment group demonstrated significantly decreased prick skin test wheal size, decreased basophil responsiveness to peanut stimulation, increased peanut-IgG₄ and decreased IL-5 levels after 12 months. Peanut-IgE 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 4182 active doses, 11 (0.3%) required treatment with oral antihistamine; one 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 eight children with cow's milk allergy.⁵⁶ Following an initial positive oral milk challenge, children started SLIT with 0.1 mL of milk for the first two weeks, increasing by 0.1 mL every 15 days until 1 mL/day was taken. Milk was kept in the mouth for 2 minutes and then spit out. Seven children completed the protocol; one child withdrew due to continued oral symptoms. Following 6 months of treatment, the threshold dose of milk increased from a mean of 39 mL at baseline to 143 mL ($P < 0.01$).

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 with a maintenance dose of 50 µg Pru p 3 for 6 months.⁵⁷ 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 following 6 months of SLIT compared to pre-SLIT threshold doses. In contrast, the placebo-treated subjects experienced no significant changes in their eliciting doses of Pru p 3. Specific IgE to recombinant Pru p 3 increased both in the active ($P < 0.001$) and placebo ($P = 0.03$) groups, although the increase remained only significant at 6 months in the active group (active 4.2, $P < 0.001$; placebo 4.0, $P = 0.08$, T -test). IgG₄ to

native Pru p 3 increased significantly in the active group ($P = 0.007$) but not in the placebo group ($P = 0.2$). 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.^{58, 59}

Epicutaneous immunotherapy (EPIT)

An alternative route of allergen delivery is *via* an epicutaneous patch. In a small pilot study, 18 children (mean age 3.8 years, range 10 months to 7.7 years) with cow milk allergy were randomized 1:1 to receive active EPIT or placebo.⁶⁰ Cow 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 skimmed milk powder or 1 mg glucose as placebo) via the skin patch per week for three 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 three months; there was no change in the placebo group. There were no significant changes in cow 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, one child had repeated episodes of diarrhea following EPIT. This small pilot study suggests that further investigation of EPIT for food allergy is warranted.

IMMUNOTHERAPY WITH MODIFIED RECOMBINANT ENGINEERED FOOD PROTEINS

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.⁶¹ (Table III) Modified food allergens may be combined with bacterial adjuvants (such as heat-killed *Listeria monocytogenes*, HKLM, or heat-killed *E. coli*, HKE) to enhance the Th1-skewing effect and decrease the Th2-skewing effect. Peanut-allergic C3H/HeJ mice were treated subcutaneously 10 weeks following sensitization with a mixture of the recombinant, modified major peanut allergens and HKLM (m Ara h 1-3 plus HKLM).⁶² All mice in the sham-treated group developed anaphylactic symptoms, whereas only 31% of mice in the m Ara h 1-3 plus HKLM group developed mild anaphylaxis during a post-treatment oral peanut challenge. In subsequent studies, a non-pathogenic strain of *Escherichia coli* containing the modified peanut proteins was used as an adjuvant and the vaccine was administered via the oral, nasal, subcutaneous and rectal route. Oral delivery was not effective, presumably due to breakdown of the peanut-containing *E. coli*. Although nasal and subcutaneous routes were effective, the rectal delivery was selected for further study because of safety concerns, since non-pathogenic *E. coli* bacteria reside in the colon. Peanut-allergic C3H/HeJ mice received 0.9 (low dose), 9 (medium dose), or 90 (high dose) µg of heat-killed *E. coli* expressing modified proteins Ara h 1-3 (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 following the final vaccine dose, and then at monthly intervals for two more months. After the first peanut challenge, all 3 doses of HKE-MP123 and the HKE-V-treated groups had reduced severity of anaphylaxis ($P < 0.01$, 0.01, 0.05, 0.05, respectively) compared with the sham-treated group. However, only the medium- and high-dose HKE-MP123-treated mice remained protected for up to 10 weeks following treatment. Peanut specific-IgE levels were

significantly lower in all HKE-MP123-treated groups ($P < 0.001$); they 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 ($P < 0.01$, 0.001, 0.001, and 0.001, respectively). IFN- γ and TGF- β synthesis were significantly increased ($P < 0.00001$ and 0.01, respectively) compared with 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 the future studies, probiotic bacteria may also be used as bacterial adjuvants to avoid the concerns of excessive Th1 stimulation by killed pathogenic bacteria.⁶⁴

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 two IgE molecules.⁶⁵ Immunization with bacterial plasmid DNA (pDNA) that encodes specific antigens can induce prolonged humoral and cellular immune Th1 responses. The Th1 effect is mediated by immunostimulatory sequences (ISSs) consisting of unmethylated cytosine and guanine motifs (CpG motifs) in the bacterial pDNA backbone. Intramuscular immunization of naïve AKR/J (H-2^K) and C3H/HeJ (H-2^K) mice with pDNA encoding Ara h 2 prior to intraperitoneal peanut sensitization had a protective effect in AKR/J mice, but induced anaphylactic reactions in C3H/HeJ mice following peanut challenge.⁶⁷ In another study, oral chitosan-embedded Ara h 2 had a protective effect in AKR mice.⁶⁸ These studies raise concern that the effect of pDNA-based immunotherapy may be strain-dependent and not universally effective in reversing IgE-mediated food hypersensitivity in man.

Synthetic immunostimulatory oligodeoxynucleotides containing unmethylated CpG motifs (ISS) 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 to sham controls.⁶⁹ Similarly, intradermal immunization with a mixture of ISS and β -galactosidase (β -gal) provided protection against fatal anaphylaxis induced by intraperitoneal β -gal sensitization and challenge that was comparable to protection provided by immunization with the pDNA-encoding β -gal.⁷⁰ Protection was associated with an increase in IgG_{2a}/IFN- γ and a decrease in IgE, IL-4, and IL-5. ISS-linked allergen immunization may have a prophylactic effect against food allergy, however, the ability to reverse established food allergy remains to be determined.

Other novel therapeutic approaches that may be utilized to treat food allergy include human immunoglobulin Fc-Fc fusion proteins that cross-link the high affinity Fc ϵ RI and low affinity Fc γ RIIb on mast cells and basophils leading to inhibition of degranulation.^{71, 72, 73} Since many major food allergens have been identified, this approach might be applied to food allergy therapy. C-type lectin receptors on dendritic cells, SIGNR-1 (also called CD209b) may play a role in promoting oral tolerance development and thus preventing food-induced anaphylaxis.⁷⁴ Mice sensitized with mannoside-conjugated BSA (Man₅₁-BSA) were protected from anaphylaxis during an oral challenge with BSA and Man₅₁-BSA whereas mice sensitized with BSA alone developed significant allergic symptoms during oral challenge with BSA. Man₅₁-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. 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 NON-SPECIFIC THERAPY

Humanized monoclonal anti-IgE

Humanized monoclonal mouse 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 Fc_εRI receptors expressed on the surface of mast cells and basophils and low affinity Fc_εR2 receptors expressed on B cells, dendritic cells and intestinal epithelial cells. With the decrease in free IgE molecules due to anti-IgE therapy, the expression of Fc_εRI receptors on mast cells and basophils is down-regulated resulting in decreased activation and release of histamine and other inflammatory mediators.⁷⁵ In addition, anti-IgE inhibits IgE-facilitated antigen uptake by B cells and antigen-presenting cells and may inhibit IgE antibody synthesis.

A multi-center clinical trial assessed the effect of humanized monoclonal anti-IgE mouse IgG₁ antibody (Hu-901) in 84 peanut-allergic adults.⁷⁶ (Table IV) Peanut allergy was confirmed by double-blind placebo controlled oral peanut challenges and the threshold dose of peanut protein eliciting objective symptoms was established. Subjects were randomized 3:1 to receive either humanized monoclonal antibody Hu-901 at 3 different doses (150, 300, or 450 mg) or placebo subcutaneously monthly for four doses. Oral peanut challenges were repeated within two to four weeks following the fourth dose of anti-IgE. The eliciting threshold dose showed an increasing trend over baseline in all three groups, with an apparent dose response, but the increase was statistically significant only in the group treated with 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 nine peanut kernels (2805 mg), $P < 0.001$ for the comparison of the 450-mg dose with placebo, and P for trend with increasing dose < 0.001 . 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 may not benefit from the anti-IgE therapy or may require higher doses for protection. A controlled trial of a different anti-IgE humanized IgG₁ antibody molecule (omalizumab [Xolair®]) in peanut-allergic patients was terminated due to the occurrence of two severe allergic reactions during the initial screening peanut challenge that raised safety concerns. Prior to 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.⁷⁷ Subjects in the Xolair arm appeared to experience a greater shift in tolerability than the placebo-treated group. ($P = 0.054$)

The combination of anti-IgE and specific allergen immunotherapy has been investigated with environmental aeroallergens, but not yet with food allergens.⁷⁸ The combination of anti-IgE and food oral immunotherapy has the hypothetical advantage of decreasing the risk of adverse reactions associated with oral immunotherapy and decreasing facilitated antigen presentation, which promotes Th2 responses. A study of anti-IgE and milk oral immunotherapy in children and adults with milk allergy is currently ongoing.

Traditional Chinese Medicine

Herbs have been used by Traditional Chinese Medicine (TCM) for many centuries, although not for food allergies. The initial study of TCM in food allergy utilized an herbal formula (FAHF-1) containing a mixture of 11 herbs, in a mouse model of peanut anaphylaxis.⁷⁹ Herbs included in FAHF-1 have been used for treating parasitic infections, gastroenteritis and asthma by TCM. FAHF-1 protected peanut-allergic mice 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 interferon- 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 nine herbs completely blocked anaphylaxis during peanut challenge up to five months following therapy.⁸⁰ This protective effect was mediated by interferon- γ produced by CD8+ T cells.^{81, 82} Each individual herb provided some degree of protection from peanut-induced anaphylaxis, but none of them offered protection that was equivalent to the complete FAHF-2 mixture of herbs, suggesting synergy among the different ingredients.

A Phase I, randomized, double-blinded, 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.⁸³ Two patients (1 in the FAHF-2 group and 1 in the placebo group) reported mild gastrointestinal symptoms. Serum interleukin-5 levels decreased in the active treatment group following 7 days of treatment with FAHF-2. *In vitro*, supernatant levels of IL-5 decreased whereas interferon- γ and IL-10 increased in allergen-stimulated peripheral blood mononuclear cells cultured with FAHF-2. A phase II, extended safety and efficacy trial is currently enrolling subjects 12-45 years 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.⁸⁴

In a mouse model of shrimp anaphylaxis, oral administration of a mixture of probiotics significantly reduced symptom scores and histamine release in the feces following 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 IL-27 mRNA expression, IL-10, TGF- β and IFN- γ tissue content were up-regulated.⁸⁵

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 develop IgE-mediated responses to food and environmental allergens.⁸⁶ 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.⁸⁷ Other studies have not replicated this finding.^{88, 89} 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 atopy⁹⁰ the cumulative prevalence of atopic dermatitis at 1 year of age was reportedly 5.7% infants in the prebiotic group compared to 9.7% infants in the control group ($P=0.04$). However a double-blind, randomized, placebo-controlled trial in 119 infants with CMA, treated with a mix of two probiotics for 12 months showed no benefit for CMA.⁹¹ There was no difference in the cumulative percentage of tolerance to CM 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**

Lactococcus lactis transfected to secrete murine IL-10 (*L. lactis*-IL-10) was administered to young mice prior to oral sensitization with β -lactoglobulin and cholera toxin.⁶⁴ Pre-treatment with *L. lactis*-IL-10 diminished anaphylaxis severity and inhibited serum β -lactoglobulin-IgE and IgG₁, and increased the production of β -lactoglobulin-IgA in the gut. *L. lactis*-IL-10 induced IL-10 secretion by Peyer's patch cells in the gut and increased plasma IL-10 titers.

Intranasal co-administration 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 Th2-to Th1-immune response.⁹²

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

Toll-like receptors

Signaling via Toll-like receptor 9 (TLR9) induces mucosal and systemic Th1 immune responses. Oral administration of a synthetic TLR9 agonist resulted in decreased gastrointestinal inflammation and protection from peanut-anaphylaxis in a mouse model of peanut allergy.⁹³ The protective effect included decreased levels of peanut-specific IgE and IgG₂ antibodies; protection was observed both when TLR9 agonist was administered during as well as following 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.⁹⁴⁻⁹⁶ In a mouse model of food allergy, helminth infection was reportedly protective against IgE-sensitization and anaphylaxis by stimulating IL-10.^{97, 98} Helminth *Trichuris suis* has been shown to be safe and beneficial in clinical trials of ulcerative colitis and Crohn's disease.^{99, 100} Although the approach of controlled helminthic infection is controversial, *T.suis* ova therapy efficacy in inflammatory bowel disease and the data from mouse models of food allergy provide a logical rationale for extending the investigation into food allergy. However, a recent study of *Trichuris suis* ova therapy in adults with allergic rhinitis found no beneficial effect on symptoms scores, days without symptoms, total histamine, grass-specific IgE, or diameter of wheal reaction on skin prick testing with grass pollen.¹⁰¹

Anti-interleukin-5 antibody (mepolizumab) in eosinophilic esophagitis

Eosinophilic esophagitis (EoE) is a disorder of mixed pathophysiology, with both IgE- 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 interleukin-5 (IL-5) in the accumulation of eosinophils in the esophageal tissue, treatment with a monoclonal anti-IL-5 antibody was investigated in a randomized, placebo-controlled, double-blinded trial.¹⁰² 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%) following the first dose ($P = 0.03$), 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.¹⁰³ In fact, three large cohort studies have provided compelling evidence that early introduction of peanut, milk, and egg into an infant's diet may decrease the risk of IgE-mediated allergy to those foods.¹⁰⁴⁻¹⁰⁶ In addition, epidemiological observations and studies in animal models have highlighted the potential for sensitization to peanut and egg white through cutaneous contact. This route favors a Th2-skewed immune response and specific-IgE production, which suggests a need for early oral introduction to counter the effect of cutaneous exposure.¹⁰⁷⁻¹¹⁰ However, in a recent study of infants at high risk of developing 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.¹¹¹ 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 food allergy. Among the plethora of novel approaches, the strategies most likely to advance into clinical practice include Chinese herbal formula FAHF-2 and oral immunotherapy alone or in combination with anti-IgE antibody. 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 food-allergic patients. 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.

Abbreviations

Anti-IgE	Anti-immunoglobulin E
Anti-IL-5	-interleukin-5
DBPCFC	Double-blind, placebo-controlled food challenge
EMP 1, 2, 3	<i>Escherichia coli</i> expressing modified peanut major allergens Ara h 1, 2, 3
EPIT	Epicutaneous immunotherapy
FAHF	Food Allergy Herbal Formula
IT	Immunotherapy
OIT	Oral immunotherapy
SLIT	Sublingual immunotherapy
TCM	Traditional Chinese Medicine

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FIG 1.
Approaches to food allergy immunotherapy

FIG 2.
Patterns of responses to the food oral immunotherapy

TABLE I

Significance of sequential IgE-binding epitopes in egg white, cow milk, and peanut

	Patient Population & Methods	Results
Egg white ovomucoid		
<i>Cooke & Sampson</i> 1997 ¹⁶	Children with persistent egg allergy and atopic dermatitis; ovomucoid dodecapeptides overlapping by 10 amino acid (AA) 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.
<i>Jarvinen</i> 2007 ²⁰	11 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 range of egg-specific IgE levels, but none of the patients with transient egg allergy had IgE antibodies against these epitopes of ovomucoid: AA 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		
<i>Jarvinen</i> 2001 ¹⁸	10 patients with persistent milk allergy and 10 patients who subsequently outgrew their milk allergy; 25 decapeptides of alpha (s1)-casein, alpha (s2)-casein, kappa-casein, alpha-lactalbumin, and beta-lactoglobulin, comprising the core epitopes, synthesized on a SPOTS membrane; Sera from individual patients were used for immunolabeling	Five IgE-binding epitopes (2 on alpha (s1)-casein, 1 on alpha (s2)-casein, and 2 on kappa-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 (AA 123-132 on alpha (s1)-casein, AA 171-180 on alpha (s2)-casein, and AA 155-164 on kappa-casein) were identified in all patients with persistent milk allergy.
<i>Wang</i> 2010 ²³	33 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)		
<i>Shreffler</i> 2004 ²¹	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 one of the recombinant allergens; 87% had detectable IgE to sequential epitopes. Individual patients had significant heterogeneity in the number and patterns of epitope recognized. High epitope diversity was found in patients with a history of more severe allergic reactions.
<i>Flintermann</i> 2008 ²²	24 peanut-sensitized children and 6 atopic controls; Specific IgE and IgG4 binding to 419 overlapping 15-AA peptides representing the sequence of recombinant Ara h 1, Ara h 2, and Ara h 3 was analyzed with microarray immunoassay.	Peanut-sensitized sera bound significantly more IgE and IgG4 epitopes than control sera. There was a positive correlation between 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.

TABLE II

Trials in food oral immunotherapy

Study	Subjects	Success rate [^]	Immunologic changes	Side effects/comments
Mixed foods				
<i>Patriarca 2003</i> clinical trial ⁴⁴	Milk (n=29) Egg (n=15) Fish (n=11) Orange (n=2); and other ^{\$}	45/54 (83.3%)	SPT became negative after 18 months in 78%; Food-IgE decreased and food-IgG4 increased after 18 months	51% of patients experienced urticaria, emesis, diarrhea, or abdominal pain. In 9 patients (16.7%) protocol was stopped due to side effects. No differences between children and adults
<i>Morisset 2007</i> ⁴⁵ randomized clinical trial	n=141; mean age: Milk: 2.2 years Egg: 3.5 years Milk (n=57) Egg (n=84)	Milk: 89% Egg: 69%	SPT sizes and specific IgE levels were significantly decreased in children that developed tolerance to milk or egg.	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 that developed tolerance to milk or egg.
<i>Staden 2007</i> ⁴¹ randomized clinical trial	Milk (n=14) Egg (n=11) Control group (n=20)	9/25 (36%) permanent tolerance; 3/25 (12%) tolerant with regular intake (desensitized), 4/25 (16%) partial responders	Allergen-specific IgE decreased significantly both in children who developed 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 following a two month period of complete avoidance of the food. The spontaneous resolution rate of food allergy in the control group was comparable, 7/20 (35%).
Cow milk				
<i>Meglio 2004</i> ¹¹²	n=21 Age: 5-10 years	15/21 (71.4%)	SPT to BLG and CS significantly decreased at 6 months ($P<.001$), milk-IgE levels not significantly different	3/21 reacted to minimal dose of diluted milk; 3/21 tolerated only 40-80 mL milk/day; 15/21 tolerated 200 mL milk/day for 6 months; Side effect rate 13/21
<i>Skripak 2008</i> ⁴⁶ randomized, placebo controlled clinical trial; <i>Narisety 2009</i> ⁴⁷ open label follow up study	n=20; active to placebo 2:1 ratio; age 6-17 years	19 subjects completed treatment; post-OIT, the median cumulative dose of milk inducing a reaction in the active group increased from 40 mg to the median dose 5140 mg; there was no change in the placebo group, $P=.0003$.	Milk-IgE levels did not change significantly in either group. Milk-IgG levels increased significantly in the active treatment group, with a predominant milk-IgG4 increase.	The median frequency of side effects was 35% in the active group compared to 1% in the placebo group. Blinded study: Mild oral pruritus median 16% doses/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 months: 2.5-96.4% of doses per subject; >3 months: 0-79%/subject % total doses with reactions: Oral pruritus: 17% Gastrointestinal : 3.7% Respiratory: 0.9% Cutaneous: 0.8% Multisystem: 5.5%

Study	Subjects	Success rate [^]	Immunologic changes	Side effects/comments
				Epinephrine: 6 reactions in 4 subjects
<i>Longo 2008</i> ⁴⁸ <i>Randomized clinical trial</i>	n=60; active treatment n=20; untreated comparison n=30 mean age 7.9 years (5-17)	After 1 year 11/30 (36%) tolerated ≥ 150 mL milk on daily basis; 16/30 tolerated 5-150 mL milk. None of the children in the comparison group tolerated >150 mL of milk during the final food challenge, $P<.001$	Reduction in cow milk-specific IgE in 15/30 subjects treated with milk OIT; no clear changes in the untreated comparison subjects.	Three children (10%) discontinued the study due to significant respiratory or abdominal side effects; 17/30 children reported side effects at home; 17 children received oral steroids, 6 received nebulized epinephrine and 1 received intramuscular epinephrine for home reactions. Six of the comparison subjects had mild symptoms upon accidental exposures to milk during the study.
Peanut				
<i>Jones 2009</i> ⁴⁹	n=39 median age at enrollment: 57.5 months (range, 12-111 months)	Open label study, follow up for 30 months. 29/39 subjects completed (74%), 27 (27/35; 77%) ingested 3.9 g peanut protein during the final food challenge.	By 6 months, titrated skin prick tests and activation of basophils significantly decreased. Peanut-IgE decreased by 12 to 18 months, whereas IgG ₄ increased significantly. Serum factors inhibited IgE-peanut complex formation in an IgE-facilitated allergen binding assay. Secretion of IL-10, IL-5, IFN-gamma, and TNF-alpha from PBMCs increased over a period of 6 to 12 months. Peanut-specific FoxP3 (+) T cells increased until 12 months and decreased thereafter. T-cell microarrays showed down regulation of genes in apoptotic pathways.	Four subjects withdrew due to the side effects (10%); 6 withdrew due to personal reasons. Most symptoms noted during OIT resolved spontaneously or with antihistamines. Adverse reactions during the <u>build up phase</u> (% of total doses): ^{44, 46, 47} Mild oropharyngeal 69% Mild/moderate skin 62% Mild/moderate nausea or abdominal pain 44% Diarrhea/emesis 21% Mild wheezing 18% Adverse reactions during the <u>maintenance phase</u> (% of total doses) Upper respiratory 29% Cutaneous 24% Any treatment: 0.7% of home doses Epinephrine: 2 subjects (one dose each)
<i>Clark 2009</i> ⁵⁰	n=4 ; case series; ages 9-13 years	Open label study; follow up for 6 weeks on maintenance dose 800 mg 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.
<i>Blumchen 2010</i> ⁵¹	n=23; age, 3.2-14.3 years	After the rush phase, patients tolerated a median 0.15 g peanut. 22/23 patients continued with the long-term protocol. After a median 7 months, 14 (63%)	There was a significant increase in peanut-specific serum IgG(4) and a decrease in peanut-specific IL-5, IL-4, and IL-2 production by PBMCs following OIT.	In 2.6% of 6137 total daily doses, mild to moderate side effects were observed; in 1.3%, lower respiratory symptoms occurred. OIT was discontinued in 4 of 22 (18%) patients because of adverse events. No epinephrine was used for

Study	Subjects	Success rate [^]	Immunologic changes	Side effects/comments
		patients reached the protective dose 0.5 -2 g peanut. At the final DBPCFC, patients tolerated a median 1 g (range, 0.3-4 g) in comparison with 0.2 g peanut at the DBPCFC pre-OIT (range, 0.02-1 g), $P=.002$.		treatment of adverse reactions.
Egg				
<i>Buchanan 2007</i> 113	n=7 mean age 4 years (subjects with history of egg-induced anaphylaxis were excluded)	4/7 (57%)	Egg white-IgG increased significantly from baseline to 24 months ($P=.002$). Five subjects showed an overall decrease in eggwhite-IgE.	Four subjects tolerated egg challenge at the end of 24 months. Two of them reacted to a subsequent egg challenge done 3 months after treatment was stopped.

[^] Symbols: Success rate defined as regular ingestion of the tested food for at least 6 months;

^{\$} One of each Apple, 4 peach, lettuce, orange, beans and corn; Abbreviations: MILK, cow's milk; SPT, skin prick test; BLG, beta-lactoglobulin; ALA, alpha-lactalbumin; CS, casein; PBMC, peripheral blood mononuclear cells; DBPCFC, double-blind, placebo-controlled food challenge

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 <i>Li XM 2003</i> ⁶³	Up-regulation of Th1 and T- regulatory cytokine responses	Protection against peanut anaphylaxis in mice, lasting up to 10 weeks after treatment	Concern for toxicity of bacterial adjuvants, excessive Th1 stimulation, and potential for autoimmunity; heat-killed <i>E. coli</i> expressing modified peanut allergens administered rectally viewed as the safest approach for future human studies. Phase I clinical trial in adults with peanut allergy is ongoing.
Pre-clinical (murine models)			
Peptide immunotherapy <i>Li S</i> ⁶⁵	Overlapping peptides (10-20 amino-acid long) that represent the entire sequence of allergen. Binding to mast cells eliminated, T-cell responses preserved	Protection against peanut anaphylaxis in mice	Improved safety profile compared with conventional IT, does not require identification of IgE-binding epitopes
Plasmid DNA immunotherapy <i>Li XM</i> ⁶⁷	Induces prolonged humoral and cellular responses due to CpG motifs in the DNA backbone	Protection against peanut anaphylaxis in sensitized AKR/J mice, but induction of anaphylaxis in C3H/HeJ (H-2 ^K) mice; no effect on peanut-IgE antibody levels	Serious concerns regarding safety in view of strain-dependent effects in mice, concern for excessive Th1 stimulation and autoimmunity
Immunostimulatory sequences immunotherapy (ISS-ODN) <i>Srivastava 2001</i> ⁶⁹	Potent stimulation of Th1 via activation of antigen-presenting cells, natural killer cells, and B cells; increased Th1 cytokines	Protection against peanut sensitization in mice	Not shown to reverse established peanut allergy, concern for excessive Th1 stimulation, and potential for autoimmunity
Engineered recombinant peanut immunotherapy <i>Srivastava 2002</i> ⁶¹	Binding to mast cells eliminated or markedly decreased, T-cell responses comparable to native peanut allergens	Protection against peanut anaphylaxis in mice	Improved safety profile compared with conventional IT, requires identification of IgE binding sites
Human immunoglobulin Fc-Fc fusion protein <i>Zhang 2004, Kepley 2004, Zhu 2005</i> ⁷¹⁻⁷³	Fusion protein cross-links the high-affinity FcεR1 and low affinity FCγRIIb receptors on mast cells and basophils	Fusion protein inhibits degranulation of mast cells and basophils	A human gamma-allergen fusion protein, the Fc-Fc d 1 fusion protein inhibited Fc d 1-mediated degranulation in purified human basophils from cat allergic patients and blocked the allergic responses in a mouse model. A similar approach can be utilized for food allergy.
Sugar-conjugated BSA <i>Zhou 2010</i> ⁷⁴	Mannoside-conjugated BSA (Man ₅₁ -BSA) targeted lamina propria dendritic cells expressing SIGNR-1	Mice sensitized with Man ₅₁ -BSA were protected from anaphylaxis during an oral challenge with	Sugar-modified food allergens might be used to induce oral tolerance by targeting SIGNR-1 and lamina propria dendritic

Therapy	Mechanism of Action	Effects	Comments
	and promoted Cd4+ type 1 T regulatory cells	BSA and Man ₅₁ -BSA	cells.

TABLE IV

Allergen-non-specific therapy for food allergy

Therapy	Mechanism of Action	Effects	Comments
Clinical trials			
Monoclonal anti-IgE <i>Leung 2003</i> ⁷⁶	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, provides protection against peanut anaphylaxis in 75% of treated patients (highest dose group).	Subcutaneous at monthly or 2 week intervals, unknown long-term consequences of IgE elimination; food non-specific; may be utilized in the combination with specific food allergen OIT
Chinese herbs FAHF-2 <i>Wang 2009, 2010</i> ^{12, 83}	<u>Up-regulation of Th1 cytokines</u> : IFN- γ , IL-12 <u>Down-regulation of Th2 cytokines</u> : IL-4, IL-5, IL-13, decreased allergen-IgE 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) <i>Straumann 2010</i> ¹⁰²	Reduced tenascin C (P = 0.03) and transforming growth factor beta-1 (P = 0.05) expression in the esophageal epithelial layer 13 weeks after initiation of treatment.	Limited improvement of symptoms; a trend was seen between 4 and 13 weeks following initiation of mepolizumab treatment.	Well tolerated; acceptable safety profile, even at the high 1500 mg dose level. Current studies evaluate mepolizumab in children with EoE.
<i>Trichuris suis</i> ova therapy <i>Summers 2005</i> ^{99, 100}	Stimulation of IL-10 synthesis	In a mouse model of food allergy protection against food IgE-sensitization and anaphylaxis	Safe and afforded clinical improvement in Crohn's disease and ulcerative colitis; no effect in adults with allergic rhinitis
Pre-clinical (murine models)			
<i>Lactococcus lactis</i> transfected with IL-10 <i>Frossard 2007</i> ⁶⁴	Decreased serum IgE and IgG ₁ ; increased gut IgA, increased gut and serum IL-10	Pre-treatment of young mice prior to 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 mouse model, however the concept of probiotic bacteria may be applied to delivery of engineered allergens in human studies
<i>Lactococcus lactis</i> transfected with IL-12 and β -lactoglobulin <i>Cortes-Perez, 2009</i> ¹¹⁴	Decreased IgG ₁ in serum and BAL; decreased IL-4 and increased IFN- γ production by β -lactoglobulin stimulated splenocytes	Intranasal co-administration of live <i>L. lactis</i> transfected with IL-12 and β -lactoglobulin inhibited allergic reactions in mice	Probiotic bacteria engineered to deliver IL-12 and food allergen may be useful for preventing IgE-sensitization to food allergens.
Toll-like receptor 9 agonist <i>Zhu 2007</i> ⁹³	Induction of mucosal and systemic Th1 responses; decreased peanut-specific IgE and IgG ₂	Oral administration of TLR-9 agonists decreased gastrointestinal inflammation and protected mice from peanut anaphylaxis	Protective effect was observed when TLR9 agonist was administered during sensitization as well as in already sensitized mice.