

New Perspectives for Use of Native and Engineered Recombinant Food Proteins in Treatment of Food Allergy

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Food allergy is defined as an adverse reaction to food mediated by the immune system [1]. Food allergies affect 2% to 4% of adults and 6% to 8% of young children in westernized societies [2]. An estimated 12 million of Americans, including 2 million school-aged children, have food allergy. Food allergic reactions account for approximately 30,000 emergency room visits, and severe food anaphylaxis is implicated in 150 to 200 deaths per year in the United States [3]. Cow's milk, egg white, soybean, wheat, peanut, tree nuts, and fish are responsible for more than 90% of food allergies in children. In adults, peanut, tree nuts, shellfish, and fish are implicated in most severe reactions, whereas the most common food allergens are fruits and vegetables that cause oral pruritus in persons with respiratory pollen allergy [4–7]. Most childhood food allergies to cow's milk, egg white, soybean, and wheat resolve with time, whereas peanut, tree nut, fish, and shellfish allergies tend to be life-long conditions [8–10].

Diagnosis of food allergy relies on a detailed history, including type of food, amount ingested, and timing and severity of symptoms, and laboratory diagnostic tests for detecting food allergen-specific IgE antibody in the skin (using a skin prick test) or in serum (preferably using ImmunoCAP). A double-blind, placebo-controlled oral food challenge is the current gold standard for diagnosing food allergy [1,11]. Feeding the suspected food under physician supervision is indicated in an individual who has no recent (past 6–12 months) history of convincing reactions and whose laboratory

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tests are below the high predictive value for clinical reactivity (Fig. 1) [12,13]. Management of food allergy requires dietary avoidance of the implicated foods and prompt treatment of allergic reactions. Accidental food allergic reactions are very common; despite avoidance, one study showed that

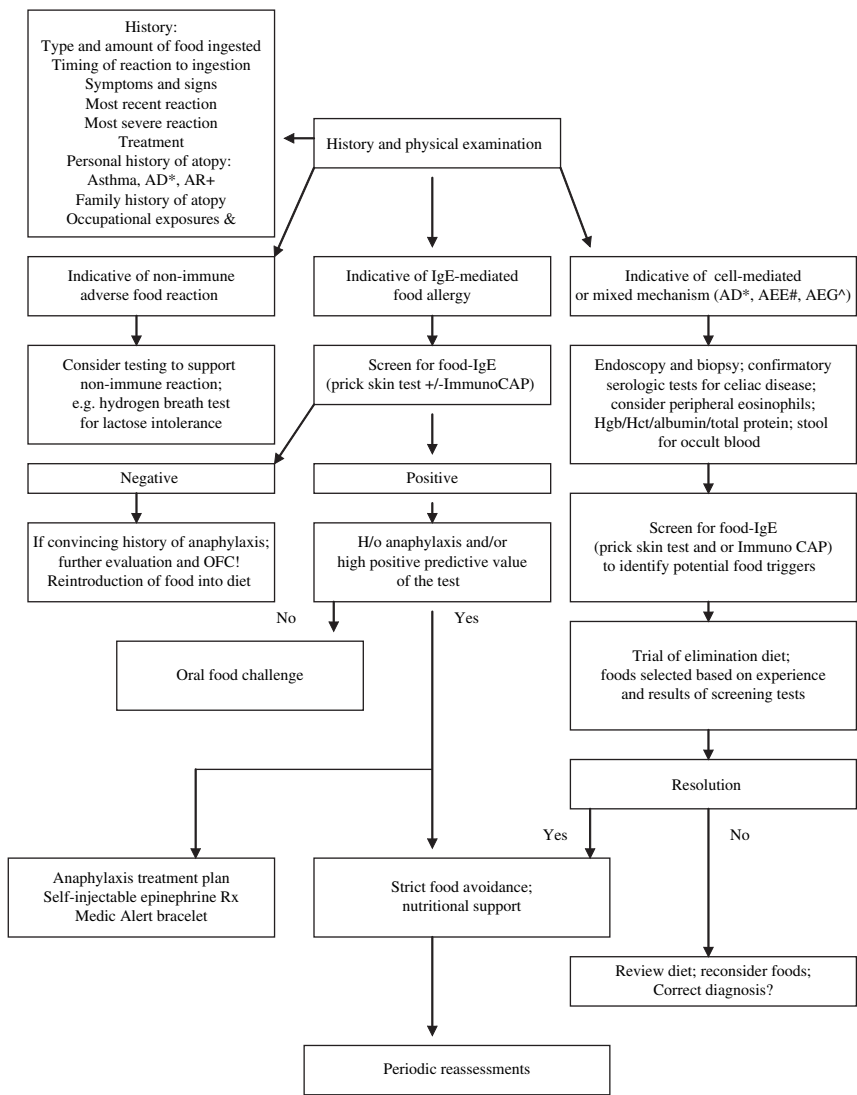


Fig. 1. A scheme of current approach to diagnosis and management of food allergy. AD*, atopic dermatitis; AR+, allergic rhinitis and occupational exposures to inhaled food dust important in bakers and millers with asthma; AEE#, allergic eosinophilic esophagitis; AEG^, allergic eosinophilic gastroenteritis; OFC!, oral food challenge.

approximately 50% of children who had peanut allergies experienced at least one accidental reaction to peanut over a 2-year period [14].

Recent epidemiologic data indicate that prevalence of peanut allergy doubled in the past 5 to 10 years in the United States, the United Kingdom, and Canada [4,5,15,16]. Currently, peanut allergy affects approximately 1% of young children, most (80%) of whom will have lifelong peanut allergy [8]. Peanut was identified as the food allergen responsible for most severe and fatal food anaphylactic reactions in the United States [17]. The reasons why peanut allergy prevalence is increasing are unknown, but considering rapid changes, environmental factors seem to play a more significant role than genetic factors. Current prevention guidelines that recommend delaying introduction of dietary peanut until age 3 years to children at risk for atopy have not been effective in decreasing the risk for peanut allergy [18].

Considering the above factors, food allergy emerged as an important target for research on curative treatment and prevention, with most efforts focusing on peanut, cow's milk, and egg allergy. This article reviews the recent developments in potential treatments for IgE-mediated food allergy using native and engineered recombinant food proteins.

Immunotherapy with native food proteins

Subcutaneous peanut immunotherapy

Subcutaneous allergen immunotherapy is used as standard and effective treatment for allergic rhinitis, asthma, and venom allergy [19]. Subcutaneous immunotherapy with peanut extract has been studied in a small number of individuals who had peanut allergy. One double-blind, placebo-controlled study treated individuals who had confirmed peanut allergy with peanut immunotherapy or placebo [20]. Objective measures of efficacy included changes in symptom score during double-blind placebo-controlled food challenge (DBPCFC) to peanut and titrated end point skin prick tests (SPT). Three subjects treated with peanut immunotherapy completed the study. These subjects showed a 67% to 100% decrease in symptoms evaluated by DBPCFC and showed a 2- to 5-log reduction in end point SPT reactivity to peanut extract. One subject treated with placebo completed the study. This subject had essentially no change in DBPCFC symptom scores or SPT sensitivity to peanut. Two other subjects treated with placebo underwent a second SPT. These subjects experienced a 1- to 2-log increase in skin test sensitivity to peanut. All subjects treated with peanut immunotherapy reached maintenance dose, and the rate of systemic reactions with rush immunotherapy was 13.3%. Because of a tragic pharmacy error, one control patient in the placebo group died of anaphylactic shock.

This study provided preliminary data showing the efficacy of injection therapy with peanut extract. A follow-up study recruited 12 patients who had immediate hypersensitivity to ingestion of peanuts [21]. Six subjects

were treated with injections of peanut extract, in whom a maintenance level of tolerance was first achieved by a rush protocol and then maintained with weekly injections for at least 1 year. The other six were untreated control subjects. All patients underwent DBPCFC to peanut initially, after approximately 6 weeks, and after 1 year. All treated patients achieved the maintenance dose of 0.5 mL of 1:100 wt/vol peanut extract with the rush injection protocol. All experienced increased tolerance to DBPCFC and decreased sensitivity on titrated SPT with peanut extract, whereas the threshold to oral peanut challenge and cutaneous reactivity to peanut extract were unchanged in the untreated control subjects.

Systemic reactions were common in the treated group both during rush immunotherapy and with maintenance injections (39%). Only three patients remained tolerant of the full maintenance dose. The increased tolerance to oral peanut challenge was maintained in the three subjects treated with full maintenance doses, but the patients who required dose reduction because of systemic reactions experienced partial ($n = 2$) or complete ($n = 1$) loss of protection. In addition, no long-term follow-up data evaluated the persistence of protection after peanut immunotherapy discontinuation. Nevertheless, this pivotal clinical study showed that food allergen could be successfully used to induce oral tolerance in subjects who have food allergies if safety was improved. The authors concluded that modified peanut extract of decreased allergenicity was needed for future clinical applications.

Birch pollen immunotherapy for pollen–food allergy syndrome (oral allergy syndrome)

Cross-reactivity between birch tree pollen and allergens in raw apple is the basis for an approach that uses subcutaneous birch tree pollen immunotherapy to treat oral allergy to apple. Respiratory sensitization to pollen allergens (eg, major birch allergen, Bet v 1) that cross-react with allergens in plant foods (eg, major apple allergen, Mal d 1) results in oropharyngeal symptoms triggered by the ingestion of raw fruits and vegetables [22]. A prospective, nonrandomized, nonblinded clinical trial of birch pollen subcutaneous immunotherapy in 49 adults who had birch pollinosis and oral symptoms provoked by apple was recently reported [23]. Subjects underwent birch immunotherapy for 12, 24, or 36 months. After undergoing immunotherapy, 41 patients (84%) compared with no (0%) controls (birch-allergic adults who underwent no immunotherapy) reported a significant reduction (50%–95%) or total clearance (100%) of apple allergy symptoms ($P < .001$).

Birch immunotherapy also induced a marked reduction in skin reactivity against fresh apple in 43 patients (88%). The effect of immunotherapy was inversely related with baseline skin reactivity. In contrast, baseline birch pollen-specific or apple-specific IgE antibody levels did not correlate with

immunotherapy effectiveness on apple allergy. No control subject reported a reduction in the severity of apple allergy or showed a decrease in skin reactivity at follow-up ($P < .001$). Immunotherapy with birch pollen extracts effectively reduced clinical apple sensitivity and skin reactivity in most cases after only 1 year of treatment.

These effects were not paralleled by a similar reduction in apple-specific IgE. In a follow-up study, the duration of the effect of birch immunotherapy was evaluated in 30 patients who had birch pollen allergy who experienced resolution of apple allergy and loss of skin-test reactivity to fresh apple [24]. Symptoms and skin-test reactivity were compared at the end of the immunotherapy course and 30 months after immunotherapy was stopped. More than 50% of patients were still able to tolerate eating apple at the 30-month follow-up visit, although most showed evidence of resensitization to apple on SPT. Both studies were criticized for lack of randomized design and objective evaluation of the severity of apple allergy using DBPCFC. However, despite limitations, these studies provided evidence that birch pollen immunotherapy may provide a long-lasting improvement in apple allergy in a subset of birch allergic individuals who have oral allergy to apple. Subsequent clinical trials, in which oral allergy to apple was diagnosed with DBPCFC, confirmed the beneficial effect of birch immunotherapy on oral tolerance to apple in some patients [25,26]. Asero [27] speculated that some patients who have oral allergy syndrome may need immunotherapy doses higher than typically needed to produce improvement in birch pollen rhinitis. He also pointed out that most significant effects on oral allergy syndrome were observed in the studies that included adults monosensitized to birch tree pollen and not to other pollens.

Oral desensitization in food allergy

Oral desensitization to food or, as proposed by Niggemann and colleagues [28], specific oral tolerance induction (SOTI) is generating increasing interest as a potential approach to treating food allergy [29]. The rationale for using the oral route is that oral ingestion of a food antigen results preferentially in the active nonresponse of the immune system toward that antigen. Animal studies suggest that high-dose feeding of an antigen results in anergy or deletion of the antigen-specific T lymphocytes, whereas intermittent feedings with small doses will more likely cause activation of the regulatory T cells and mediators [30]. Although some researchers postulate that food allergy results from the failure to develop, or a breach in, oral tolerance, direct evidence in humans has not been established.

With allergy to cow's milk, peanut, or egg, experts have assumed that the major route of sensitization is through the gastrointestinal tract because these proteins are resistant to proteolytic enzymes and low gastric pH [2]. In contrast, sensitization to proteins in raw fruits and vegetables that are highly cross-reactive with pollen allergens predominantly occurs through

the respiratory tract and is primarily directed to inhaled pollen with subsequent reactivity to ingested cross-reactive food [31]. However, experimental murine models show that epicutaneous sensitization to food allergens preferentially induces Th 2 responses and results in allergic responses to inhaled and ingested food proteins [32–34].

In mice, oral tolerance induction is highly dose-dependent and differs for the allergenic proteins of peanut and egg ovalbumin [35]. Tolerance to peanut requires a significantly higher oral dose than does tolerance to ovalbumin. Low doses of peanut are more likely to induce oral sensitization and increase production of interleukin (IL)-4 and specific IgE on challenge. Retrospective epidemiologic data from the United Kingdom collected as maternal recall and subject to recall bias suggest that application of creams containing peanut oil is a risk factor for the development of peanut allergy in infants, especially in the setting of atopic dermatitis and impaired skin barrier [36]. In addition, up to 50% of children who have peanut and egg allergy experience their initial food allergic reaction to the first known ingestion of these foods, suggesting that prior exposures occurred either through unknown ingestion, such as resulting from cross-contamination of trace amounts from shared equipment, through transmission in breast milk, or potentially through an alternative route, such as skin or respiratory tract [37]. Because approximately 90% of children who have food allergy have atopic dermatitis and at least 40% to 50% of young children who have persistent moderate-to-severe atopic dermatitis have food allergy, epicutaneous sensitization to the common food allergens through impaired skin barrier may represent an important underappreciated route and provide the rationale for trials of oral tolerance induction to these foods.

Current evidence supporting oral desensitization is limited to nonrandomized clinical trials and case reports with little insight into the immunomodulatory effects of oral food desensitization (Table 1) [38–47]. In addition, because of methodology issues, the actual effects of oral desensitization versus the natural resolution of allergy to foods such as cow's milk and egg that are typically outgrown by most children is difficult to appreciate. Nevertheless, in many patients, tolerance to food seems to be achieved and maintained for up to 6 months if the food is ingested regularly [46,47]. However, in some patients who reach maintenance dose, allergic symptoms redevelop if the food is not ingested regularly, highlighting a concern as to whether oral desensitization is capable of inducing permanent tolerance [29].

In a subset of individuals, the full maintenance dose cannot be achieved because of adverse reactions, but the patients benefit from increased threshold dose of food. Patients are protected from reactions to trace amounts, and benefit from increased safety, comfort, and nutritional value when they continue to ingest the food on daily basis to guarantee maintenance of desensitization state [47]. Compared with subcutaneous allergen immunotherapy, the concept of oral immunomodulation and tolerance induction for therapy of food allergy is undeniably appealing, especially considering the

low rate of serious adverse reactions and the comfort of home-administration. However, rigorous clinical trials are necessary to fully evaluate the role of oral desensitization in the definitive treatment of food allergy. Finally, mechanistic studies are needed to understand the immunologic changes induced by oral desensitization.

Sublingual immunotherapy with food extract

Another approach to food therapy is sublingual immunotherapy with food extracts. A recent randomized, double-blind, placebo-controlled trial evaluated sublingual immunotherapy for treating hazelnut allergy using commercial hazelnut extract [48]. Adult subjects who had hazelnut allergy confirmed with DBPCFC were randomly assigned to two treatment groups: hazelnut immunotherapy [11] or placebo [1]. Patients kept the immunotherapy solution in the mouth for at least 3 minutes and then discharged it. According to a rush schedule, all patients undergoing hazelnut immunotherapy reached the planned maximum dose in 4 days under physician supervision in the hospital, followed by home administration of a daily maintenance dose of five drops of the most potent extract over 5 months (November–March). The maintenance daily dose contained 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 administered, were limited to the rush build-up phase, and were treated with oral antihistamines. Mean threshold dose of ingested hazelnut for objective symptoms increased from 2.3 g to 11.6 g ($P = .02$; active group) versus 3.5 g to 4.1 g ($P =$ not significant; placebo) at follow-up evaluation. Almost 50% of patients who underwent active treatment reached the highest dose (20 g) of hazelnut during follow-up DBPCFC compared with 9% in the placebo group. Levels of serum hazelnut-specific IgG₄ antibody and total serum IL-10 increased only in the active group, but no differences were seen in hazelnut-specific IgE antibody levels pre- and postimmunotherapy.

These preliminary data are encouraging, especially in terms of safety, but more studies are needed to determine optimal duration of immunotherapy and persistence of protective effect when immunotherapy is discontinued. In addition, immunotherapy trials with different food allergens and in different patient populations (eg, children versus adults and subjects who have well-defined anaphylactic reactivity to foods) must be conducted before the role of sublingual immunotherapy in treating food allergy can be determined.

Recombinant food proteins

In the past decade, tremendous progress was accomplished in identifying the relevant food proteins. Major food allergens, such as those in cow's milk, egg white, soybean, wheat, peanut, tree nuts, seeds, fish, and shellfish were characterized by their structure and function. Proteins cross-reactive

Table 1
Summary of current experience with oral food desensitization for IgE-mediated food allergy

Study	Foods	Subjects	Starting dose	Time to maintenance	Success rate ^a	Comments
Patriarca et al., 1984 [38] Open clinical trial	CM (8)	N = 19 Age: 5–55 years	10 drops of CM in 10 mL of water; 4 drops/d	100 mL of undiluted CM per day in 104 days	5/8	Side effects in 11/19 patients: urticaria, pruritus, emesis, angioedema, abdominal pain, rhinitis, dyspnea; patients followed up for 3–12 months; subject who had orange allergy reported resolution of allergy to plums and peaches; no insight into mechanism of desensitization
	Egg (8)		10 drops of beaten egg in 100 mL of water, 4 drops/d	120 drops of pure beaten egg per day in 90 days	6/8	
	Fish (2)		10 mL of mixed fish commercial extract (eel, sardine, codfish, anchovy) in 90 mL of water, 4 drops/d	200 g of cooked fish/day in 120 days	2/2	
	Orange (1)		Unspecified	3 months	1/1	
Patriarca et al., 1998 [39] Clinical trial	CM (6) Egg (5) Fish (2) Apple (1)	N = 14 Age: 4–14 years	Modification of previously published protocol [38]	Modification of previously published protocol [38]	12/14	All children who achieved maintenance continued to tolerate the foods at least 2–3 times per week for 3–6 years 10/14 patients experienced side effects during treatment
Bauer et al., 1999 [40]	CM	N = 1 Age 12 years	1 mL/d of 0.01% milk diluted in water	Rush protocol: dose doubled every other hour; final dose 200 mL of undiluted milk achieved in 5 days	1/1	Patient tolerated cow's milk daily for at least 6 months of follow-up

Nucera et al., 2000 [41]	Cow's milk	N = 1 Age 6 years	10 drops of milk in 10 mL of water; 4 drops per day	100 mL of undiluted cow's milk per day in 104 days	1/1	Child able to ingest CM and dairy products after 6 months; after 7 months; SPT to BLG, ALA, and CS became negative; IgE to milk proteins decreased significantly, whereas milk-IgG and IgA increased; PBMC stimulated with BLG produced significantly less IL-4 at 18 months than at baseline
Rueff et al., 2001 [42]	Celery	N = 1 Age: 49 years	0.1 mL of a commercial natural celery juice five times a day	5 mL five times daily for 3 months	1/1	At 3 months, patient tolerated 10 g of raw celery but developed flushing to 20 g; continued to ingest 25 mL per day of raw celery juice for 3 years
Patriarca et al., 2003 [46] Clinical trial	CM (29) Egg (15) Fish (11) Orange (2); and other ^b	N = 59 Age: 3–55 years	Modification of previously published protocol [38]	Modification of previously published protocol [38]	45/54 (83.3%)	51% of patients experienced urticaria, emesis, diarrhea, or abdominal pain; in 9 patients (16.7%), protocol was stopped because of side effects; no differences between children and adults; SPT became negative after 18 months in 78%; food-IgE decreased and food-IgG4 increased after 18 months
Meglio et al., 2004 [47]	CM	N = 21 Age: 5–10 years	1 drop of CM diluted 1:25 in water	200 mL undiluted CM per day over 180 days	15/21 (71.4%)	3/21 reacted to minimal dose of diluted CM; 3/21 tolerated only 40–80 CM per day; 15/21 tolerated 200 mL CM per day for 6 months; Side effect rate 13/21; SPT to BLG and CS significantly decreased at 6 months ($P < .001$), CM-IgE levels not significantly different

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Table 1 (*continued*)

Study	Foods	Subjects	Starting dose	Time to maintenance	Success rate ^a	Comments
Rolinck-Werninghaus et al., 2005 [29]	CM (1) Egg (2)	N = 3 Age: 4–12 years	0.0006 mL of CM per day; 0.01 mg egg per day at home	100 mL CM per day over 37 weeks, 2.5 g egg per day (1/2 egg) over 41–52 weeks	3/3	All patients had acute symptoms to food re-exposure after discontinuation of food for 2–14 days; the only study to rechallenge patients to food after a period of avoidance while on maintenance
Patriarca et al., 2006 [43]	Peanut	N = 1	5.6 mg peanut per day on day 1	40 g peanut per day over 7 days in the hospital	1/1	SPT to peanut became negative at 6 months, no significant changes in peanut-IgE (baseline: 2.1, 6 months: 1.5) or peanut-IgG
Pons et al., 2006 [44]	Egg white	N = 3	Not specified	Rush immunotherapy on day 1 in the hospital, then build-up over several months	3/3	Percentage of egg-specific CD4 ⁺ CD23 ^{high} T cells increased starting between 3 and 6 months, in parallel with production of IL-10 by egg white-stimulated PBMC
Buchanan et al., 2006 [45]	Peanut	N = 7 Mean age 4.4 years	Not specified	Rush phase and dose escalation in the hospital, maintenance at home	7/7 at 6 months	During rush phase 4/7 required oral antihistamine; at 6 months a mean 2.3-fold increase in peanut-IgG and mean change in peanut-IgE of 0.9-fold occurred

Abbreviations: ALA, alpha-lactalbumin; BLG, beta-lactoglobulin; CM, cow's milk; CS, casein; PBMC, peripheral blood mononuclear cells; SPT, skin prick test.

^a Success rate defined as regular ingestion of the tested food for at least 6 months.

^b One of each: apple, peach, lettuce, orange, beans, and corn.

with birch tree pollen found in foods of plant origin (eg, fruits, vegetables, legumes, and tree nuts) responsible for oral allergy syndrome were extensively studied [31]. Severity of peanut allergy was correlated with the diversity of epitopes recognized on the major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, by B cells [49–51]. Individuals allergic to cow's milk, egg, and peanut who had no IgE antibodies against certain sequential epitopes of the major allergens were found to be more likely to achieve tolerance than subjects whose IgE antibodies were directed against those epitopes [52–55].

Identification of the most relevant food allergens was followed by generation of recombinant proteins [56]. To date, a significant number of recombinant food allergens have been synthesized, including major peanut allergens (Ara h 1, 2, and 3); major allergens from English walnut (Jug r 1), hazelnut (Cor a 1.04), and cashew (Ana o 1); tropomyosins from crab, lobster, shrimp, and snail; parvalbumins from salmon, carp, and codfish; lipid transfer proteins from carrot, bell pepper, tomato, wheat, and cherry; birch Bet v 1 cross-reactive major allergens from carrot, apple, and celery; soybean glycinin; and egg white ovomucoid. Most recombinant food proteins were evaluated in regard to their ability to bind specific IgE antibody from allergic patients' sera, and some were used for skin prick testing [51,57,58]. The availability of pure recombinant allergens should enable customization of diagnostic tests and result in more precise testing for individuals who have food allergies. It should also allow for improved safety of testing procedures by eliminating contaminations and undeclared allergens in wild-type allergen extracts [59].

Animal models of food hypersensitivity

Because of ethical concerns about serious side effects of food immunotherapy, animal models were crucial in evaluating efficacy and safety of experimental therapies. Li and colleagues [60,61] developed well-characterized murine models of IgE-mediated cow's milk and peanut hypersensitivity in which subjects were sensitized orally and developed anaphylaxis after oral feeding. In the peanut model, C3H/HeJ mice were sensitized orally with freshly ground whole peanut and cholera toxin as adjuvant and challenged orally 3 and 5 weeks later with crude peanut extract [61]. Peanut-specific IgE was induced through oral peanut sensitization, and hypersensitivity reactions were provoked by feeding peanut to sensitized mice. The symptoms were similar to those seen in human subjects (Fig. 2). Ara h 1- and Ara h 2-specific IgE antibodies were present in the sera of mice with peanut allergy. Furthermore, these Ara h 2-specific IgE antibodies bound the same Ara h 2 isoforms and major allergenic epitopes as antibodies in the sera of humans who had peanut allergy. Splenocytes from mice with peanut allergy exhibited proliferative responses to Ara h 1 and Ara h 2. This murine model of peanut allergy mimics the clinical and immunologic characteristics

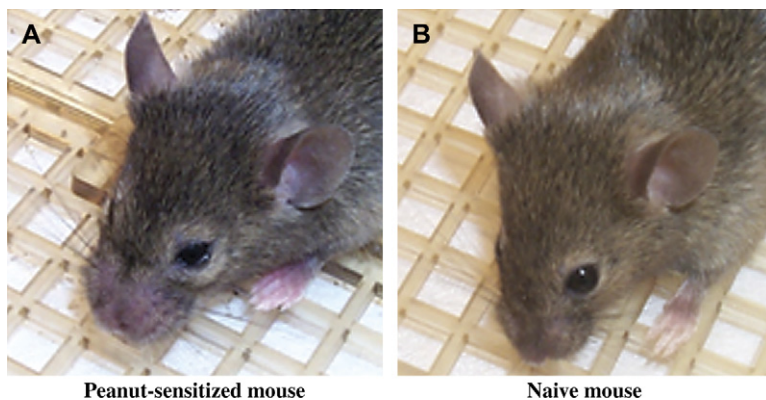


Fig. 2. Murine model of peanut anaphylaxis. (A) Peanut allergic mouse showing edema of the snout, eye, and paws, and pilar erecti after oral peanut challenge (score 3) (B) Non-peanut allergic mouse asymptomatic on feeding with peanut (score 0). C3H/HeJ mice were sensitized intragastrically with PN and cholera toxin on days 0, 7, and 14. At week 3, mice were challenged with 10 mg of crude peanut extract intragastrically. Symptoms were scored using a 0- to 5-point scale, with 0 indicating no signs of reaction and 5 indicating death. (*Reprinted from* Li XM, Srivastava K, Grishin A, et al. Persistent protective effect of heat-killed *Escherichia coli* producing “engineered,” recombinant peanut proteins in a murine model of peanut allergy. *J Allergy Clin Immunol* 2003;112(1):159–67; with permission. Copyright © 2003, American Academy of Allergy, Asthma, and Immunology.)

of peanut allergy in humans and has been used subsequently in many studies.

Immunotherapeutic approaches based on recombinant food proteins

An early study found that the intramuscular immunization of naïve AKR/J (H-2^K) and C3H/HeJ (H-2^K) mice with plasmid DNA encoding Ara h 2 before intraperitoneal peanut sensitization had some protective effects in AKR/J mice, but induced anaphylactic reactions in peanut-sensitized C3H/HeJ mice after peanut challenge [62]. Li and colleagues [62] also found no reduction in peanut-IgE antibody levels and more severe anaphylactic symptoms after oral challenge in peanut-allergic mice that were treated with pDNA-expressing Ara H 2. However, in another study, oral chitosan-embedded Ara h 2 had a protective effect in preventing sensitization in AKR mice [63]. These data indicate that pDNA-based immunotherapy may not be effective in reversing established IgE-mediated hypersensitivity.

Conjugated allergen administration with synthetic immunostimulatory oligodeoxynucleotides containing unmethylated CpG motifs (ISS) was more effective than a mixture of antigen and ISS in suppressing allergic airway responses, probably because of the enhanced dendritic cell uptake of ISS-allergen. Srivastava and colleagues [64] explored the concept of ISS potentiation of Th 1 responses to food allergens through immunizing

C3H/HeJ mice intradermally with either ISS-linked Ara h 2, or ISS-linked Amb a 1 as a control. Four weeks after immunization, mice were intragastrically sensitized with peanut and challenged with Ara h 2 5 weeks later. Mice treated with ISS–Ara h 2 did not develop symptoms and had significantly lower plasma histamine levels after oral challenge compared with those treated with ISS–Amb a 1.

Nguyen and colleagues [65] found that intradermal immunization with a mixture of ISS and β -gal, but not with ISS alone or β -gal alone, provided protection against fatal anaphylaxis induced by intraperitoneal β -gal sensitization and challenge that was associated with an increase in IgG2a/interferon (IFN)- γ and a reduction in IgE/IL-4 and IL-5. This effect was comparable to immunization with the pDNA-encoding β -gal. These results suggest that antigen–ISS immunization may have a prophylactic effect against allergy. However, the ability to reverse established food allergy remains to be determined.

Recombinant engineered food proteins

Immunotherapy with native peanut was found to have an unacceptably high rate of adverse reactions to immunotherapy injections, but the concept of using immunomodulation with peanut protein to increase oral tolerance to peanut was confirmed [20,21]. Subsequent studies concentrated on generating hypoallergenic recombinant peanut proteins that lost the ability to interact with IgE antibodies directed against native peanut but retained the ability to interact with T cells. These engineered recombinant peanut proteins were expected not to activate mast cells and thus ensure an improved safety profile.

In the initial study, peanut major allergen Ara h 1 was cloned and characterized as a member of the vicillin storage protein family [66]. RNA isolated from peanut species (Florunner) was used to construct an expression library through western blot analysis for screening with serum IgE from patients who had peanut allergy. Several clones were selected with intense binding to sera of patients who had peanut allergy. When incubated with these sera, allergens from 94% of patients that bound to wild-type Ara h 1 also bound to recombinant Ara h 1.

Subsequently, two additional major peanut allergens were cloned and characterized: Ara h 2 and Ara h 3 [67–69]. The IgE-binding epitopes of these allergens have been determined. Amino acids critical to each epitope were identified, and site-directed mutagenesis of the allergen cDNA clones was used to produce engineered recombinant allergens. Engineered recombinant peanut allergens were then analyzed through immunoblotting with peanut-allergic patients' sera [70]. Fifty-seven patients were tested for IgE binding to Ara h 1, 52 patients for Ara h 2, and 52 patients for Ara h 3. IgE binding to engineered recombinant allergens ranged from 5.4% to 125% of wild-type Ara h 1, 0% to 99.8% of wild-type Ara h 2, and 19.3% to 141%

of wild-type Ara h 3. Peripheral blood mononuclear cells (PBMC) from subjects who had peanut allergy were incubated with engineered recombinant peanut allergens and proliferation was assessed through the incorporation of radioactive thymidine into the DNA of dividing cells. PBMCs from 12 individuals who had peanut allergy were tested for each wild-type and engineered recombinant allergen. The average stimulation index produced by the engineered recombinant allergens in comparison to its wild-type counterpart was 72% for Ara h 1, 104% for Ara h 2, and 72% for Ara h 3.

Subsequently, wild-type Ara h 2 and engineered recombinant Ara h 2 were compared in their ability to interact with T cells, bind IgE, and release mediators from passively sensitized RBL-2H3 cell line. Multiple T-cell epitopes were identified on Ara h 2. Amino acid regions of Ara h 2, 11–35, 86–125, and 121–155 contained most peptides that interact with T cells from patients who had peanut allergy. Wild-type and engineered recombinant Ara h 2 stimulated proliferation of T cells from patients who had peanut allergy to similar levels. In contrast, engineered recombinant Ara h 2 greatly reduced IgE-binding capacity and released significantly lower amounts of β -hexosaminidase, a marker of IgE-mediated RBL-2H3 degranulation, compared with the wild-type allergen [71]. These studies showed that the engineered recombinant peanut allergens retained the ability to interact with T cells while they lost the ability to interact with IgE antibodies directed against native peanut proteins, supporting their use in trials of peanut immunotherapy.

Recombinant major apple allergen (rMal d 1) with five-point mutations introduced by site-directed mutagenesis was recently evaluated in 14 adults who had birch tree pollen and apple allergy [58]. IgE reactivity to mutated rMal d 1 was twofold lower than that of the wild-type molecule (95% CI; 1.7–2.4). Radioallergosorbent test (RAST) inhibition showed a 7.8-fold decrease in IgE-binding capacity (95% CI; 3.0–12.6). The biologic activity of mutant rMal d 1 assessed with SPT and basophil histamine release decreased 10- to 200-fold. In addition, DBPCFC in two subjects confirmed lower allergenicity of mutant rMal d 1 compared with wild-type rMal d 1, as measured by increased threshold dose, inducing symptoms, and maximum severity of reaction at the peak dose. This study was the first to show hypoallergenicity of engineered recombinant food allergen through an oral food challenge. These results are encouraging, but must be interpreted cautiously, because point mutations may result in formation of new allergenic epitopes and paradoxically increase allergenicity of mutated allergens for some patients [70].

Engineered recombinant peanut immunotherapy

In vivo efficacy of the engineered recombinant peanut proteins was tested in the murine model of peanut anaphylaxis [72]. Mice were sensitized to whole peanut and then desensitized through intranasal administration of engineered recombinant Ara h 2 (three doses per week for 4 weeks).

Desensitization with the engineered recombinant Ara h 2 protein suppressed synthesis of Ara h 2-IgE and resulted in significantly decreased symptoms on oral peanut challenge compared with a control group treated with wild-type Ara h 2.

Engineered recombinant peanut proteins mixed with bacterial adjuvants immunotherapy

Bacteria are potent stimulants of Th 1 immune responses and increase IFN- γ production. Heat-killed *Listeria monocytogenes* (HKLM) was shown to reverse established allergic airway hyperreactivity in mice [73]. In a dog model, a single subcutaneous treatment with a mixture of HKLM, milk, and wheat significantly reduced immediate skin test reactions and prevented anaphylactic symptoms on oral food challenge [74]. Li and colleagues [75] tested the efficacy of a mixture of HKLM and modified peanut proteins (Ara h 1, Ara h 2, and Ara h 3). Peanut-allergic C3H/HeJ mice were treated 10 weeks after sensitization with a mixture of the three major peanut allergens and HKLM (modified [m]Ara h 1–3 plus HKLM) administered subcutaneously. All mice in the sham-treated groups exhibited anaphylactic symptoms with a median symptom score of 3, whereas only 31% of mice in the mAra h 1–3 plus HKLM group developed mild anaphylaxis, with a low median symptom score of 0.5 on a scale of 0 to 5. Changes in core body temperature, bronchial constriction, plasma histamine, and peanut-specific IgE levels were all significantly reduced. This protective effect was markedly more potent than in the group treated with mAra h 1–3 protein

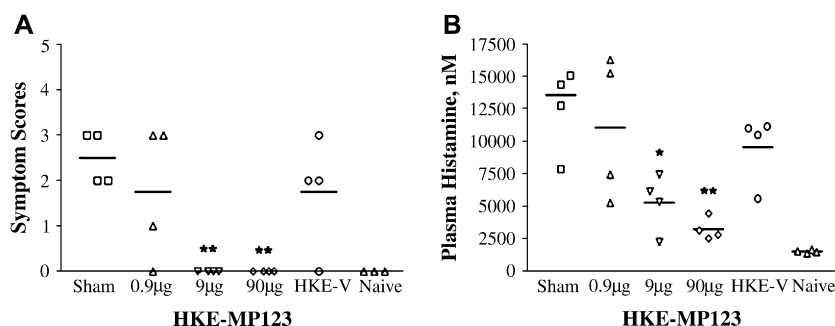


Fig. 3. Persistent protection against peanut-induced anaphylactic reactions by HKE-MP123. Mice were challenged at 10 weeks after the last HKE-MP123 treatment. (A) Anaphylactic symptom scores were determined 30 minutes after challenge. Each point indicates an individual mouse. Bars indicate the median of four mice in each group. (B) Histamine plasma levels were measured in blood samples obtained 30 to 40 minutes after the challenge. Data are means $\pm$ SEM for each group of four mice. * $P < .05$, and ** $P < .01$ versus sham. (Reprinted from Li XM, Srivastava K, Grishin A, et al. Persistent protective effect of heat-killed *Escherichia coli* producing "engineered," recombinant peanut proteins in a murine model of peanut allergy. *J Allergy Clin Immunol* 2003;112(1):159–67; with permission. Copyright © 2003, American Academy of Allergy, Asthma, and Immunology.)

Table 2
Comparison of native, recombinant, and engineered recombinant allergen immunotherapy for food allergy

Therapy	Mechanism of action	Effects	Comments
Conventional peanut IT	Altered T-cell responses, up-regulation of suppressor cells in allergen IT	Increased oral peanut tolerance	Subcutaneous injections of gradually increasing doses of allergen, unacceptably high rate of serious adverse events
Birch pollen IT for oral allergy to apple	Marked reduction in skin test to raw apple; IT inversely correlated with baseline skin test but not with serum apple or birch-IgE	Significant reduction or total resolution of oral allergy to raw Golden Delicious apple in a subset of patients undergoing IT for at least 12 months	Clinical effect lasting for up to 30 months after discontinuation in > 50% of patients
Oral desensitization	Presumed oral tolerance induction; decreased skin test reactivity, increased serum food-IgG/IgG4, no change in food-IgE, increased food-specific CD4+CD23 high T cells, increased IL-10 on food antigen stimulation	Tolerance to regular servings of food in most subjects (70%–80%) maintained as long as food ingested on regular basis for up to 6 months; in a subset increased threshold dose for clinical reactions	Up to 50% experience systemic side effects; some patients require uninterrupted ingestion of food to maintain desensitized tolerant state; rigorous clinical trials necessary to determine safety and efficacy
Sublingual IT with hazelnut extract	Presumed oral tolerance induction; increased serum hazelnut-IgG4 and total IL-10 level, no change in hazelnut-IgE	Increased oral hazelnut tolerance	Systemic reactions in only 0.2% of doses during build-up phase, treated with oral antihistamines; rigorous clinical trials necessary to determine safety and efficacy

Plasmid DNA–based IT	Induces prolonged humoral and cellular responses because of 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 Th 1 stimulation and autoimmunity
Immunostimulatory sequences (ISS-ODN)	Potent stimulation of Th 1 through activation of antigen-presenting cells, natural killer cells, and B cells; increased Th 1 cytokines	Protection against peanut sensitization in mice	Not shown to reverse established peanut allergy, concern for excessive Th 1 stimulation, and potential for autoimmunity
Engineered recombinant peanut IT	Binding to mast cells eliminated, 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
Heat-killed bacteria mixed with or expressing engineered recombinant peanut proteins	Potentiation of Th 1 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 Th 1 stimulation, and potential for autoimmunity; heat-killed <i>Escherichia coli</i> expressing modified peanut allergens administered rectally viewed as the safest approach for future human studies

Abbreviation: IT, immunotherapy.

alone. HKLM alone showed no protective effect. Reduced IL-5 and IL-13 and increased IFN- γ levels were observed only in peanut-stimulated splenocyte cultures from mice treated with mAra h 1–3 plus HKLM. These results showed that immunotherapy with modified peanut proteins and HKLM was effective in treating peanut allergy in this model, and might be a potential treatment approach.

Because of safety concerns about using potentially pathogenic bacteria in humans, a nonpathogenic strain of *Escherichia coli* was selected as a bacterial adjuvant. Subsequent studies investigated heat-killed *E coli* (HKE) expressing modified peanut proteins administered subcutaneously and found that lower doses were required for desensitization [76]. However, considering potential complications resulting from subcutaneous administration in humans, further studies focused on the vaccine administered per rectum. Because nonpathogenic *E coli* bacteria reside in the colon, experts assumed that rectal delivery would provide superior safety regarding possible infectious complications and severe adverse reactions. In addition, the rectal route is noninvasive and could be safely used in young children who would probably be the target for this vaccine.

The long-term immunomodulatory effect of HKE producing mutated Ara h 1, 2, and 3 (HKE-MP123) administered rectally was investigated in a murine model of peanut anaphylaxis [77]. Peanut-allergic C3H/HeJ mice received 0.9 μg (low-dose), 9 μg (medium-dose), or 90 μg (high-dose) HKE-MP123 administered rectally; HKE-containing vector (HKE-V) alone; or vehicle alone (sham) weekly for 3 weeks. Mice were challenged 2 weeks later. Second and third challenges were performed at 4-week intervals. After the first challenge, all three groups treated with HKE-MP123 and HKE-V showed reduced symptom scores ($P < .01$, $.01$, $.05$, and $.05$, respectively) compared with the sham-treated group. Only the medium- and high-dose HKE-MP123-treated mice remained protected for up to 10 weeks after treatment, accompanied by a significant reduction of plasma histamine levels compared with sham-treated mice ($P < .05$ and $.01$, respectively) (Fig. 3). IgE levels were significantly lower in all HKE-MP123-treated groups ($P < .001$), and were most reduced in the high-dose HKE-MP123-treated group at each challenge. In vitro IL-4, IL-13, IL-5, and IL-10 production by peanut-stimulated splenocytes of mice treated with high-dose HKE-MP123 were significantly decreased ($P < .01$, $.001$, $.001$, and $.001$, respectively), and IFN- γ and transforming growth factor β production were significantly increased ($P < .001$ and $.01$, respectively) compared with sham-treated mice at the last challenge. Treatment with rectal HKE-MP123 can induce long-term down-regulation of peanut hypersensitivity, which might be secondary to decreased antigen-specific Th 2 and increased Th 1 and T-regulatory cytokine production. The rectal vaccine is currently being standardized in preparation for a phase I human clinical trial. Table 2 compares experience with native, recombinant, and engineered recombinant allergen immunotherapy for food allergy.

Non-allergen-specific approaches to food allergy treatment

In addition to research focusing on immunotherapy with native and engineered recombinant food proteins, alternative approaches to food allergy therapy have been investigated, including monoclonal anti-IgE therapy, Chinese herbs, and cytokine therapy [78–82].

Summary

Food allergy is a serious medical problem without definitive treatment at this time. Intense research focuses on severe peanut allergy. Recombinant peanut major allergens engineered to lose IgE-binding capacity mixed with *E coli* showed great promise in a murine model of peanut anaphylaxis. Rectal vaccine containing *E coli* expressing engineered recombinant major peanut allergens Ara h 1, 2, and 3 is in preparation for first human clinical trials. Oral desensitization and sublingual immunotherapy with food extracts represent another approach that is being actively explored. Novel therapies must be evaluated carefully regarding safety and long-lasting effect on oral food tolerance before being applied in clinical practice. Diversity of approaches and promising preliminary results bring hope for patients who have food allergy.

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