Treatments for food allergy: how close are we?

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Abstract Food allergy continues to be a challenging health problem, with prevalence continuing to increase and anaphylaxis still an unpredictable possibility. While improvements in diagnosis are more accurately identifying affected individuals, treatment options remain limited. The cornerstone of treatment relies on strict avoidance of the offending allergens and education regarding management of allergic reactions. Despite vigilance in avoidance, accidental ingestions and reactions continue to occur. With recent advances in the understanding of humoral and cellular immune responses in food allergy and mechanisms of tolerance, several therapeutic strategies for food allergies are currently being investigated with the hopes of providing a cure or long-term remission from food allergy.

Keywords Food · Allergy · Anaphylaxis · Treatment · Tolerance

Introduction

Food allergy is an adverse immunologic response to food allergens that is potentially life-threatening. Recent estimates indicate that up to 8 % of children and 3–4 % of adults in Westernized countries are affected [1–3]. The most common food allergens causing reactions in children include milk, egg, wheat, soy, peanuts, tree nuts, fish and shellfish. While allergies to milk, egg, wheat and soy are commonly outgrown in childhood, allergies to peanut, tree nuts, fish and shellfish often persist in adulthood. Unfortunately, there are no treatments that can cure or provide protection from food-allergic reactions. Current standard of care consists of education about allergen avoidance and provision of emergency medications (e.g., self-injectable epinephrine) in case of allergic reactions. While this approach is generally effective, avoidance can be very difficult, resulting in a significant negative impact on

quality of life [4]. Furthermore, food-allergic reactions are unpredictable and can be fatal [5–8]. Therefore, the development of treatments for food allergies is a priority in food allergy research. Here, we discuss several strategies, both allergen-specific and allergen-non-specific (Tables 1, 2), that are currently being investigated with the aim of long-term treatment and possible cure for established food allergies.

Allergen-specific immunotherapy

Subcutaneous immunotherapy (SCIT)

Although the first report of successful SCIT for food allergy was published in 1930 [9], no new reports emerged until the 1990s when SCIT for peanut allergy was reported [10]. In this double-blind, placebo-controlled trial of rush immunotherapy in peanut-allergic patients, 3 subjects completed the study and had a 67–100 % decrease in symptoms induced by double-blind, placebo-controlled food challenge (DBPCFC) following treatment. There was also a 2–5-log reduction in end point skin prick tests (SPT) to peanut extract in these subjects. Only one placebo

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Table 1 Allergen-specific therapies undergoing human clinical trials

Oral immunotherapy (OIT) with natural proteins—several studies for milk, egg and peanut, including double-blind, placebo-controlled studies [13–27]
Oral immunotherapy (OIT) with heat-denatured proteins—longitudinal studies completed for milk and egg [28, 29, 33]
Sublingual immunotherapy (SLIT)—several studies for kiwi, hazelnut and peanut [37–41]
Epicutaneous immunotherapy (EPIT)—pilot study completed for milk [42]
Immunotherapy with modified recombinant proteins—phase 1 trial for peanut [48]

Table 2 Allergen-non-specific therapies

Anti-IgE treatment (in combination with immunotherapy or alone) [57–59]
Traditional Chinese medicine (FAHF-2) [60–67]
Anti-IL5 (for EoE) [71–73]
TLR agonists (e.g., TLR9, TLR5) [74–76]
Probiotics [80–82]
Helminth infection [85]

subject completed the study; there was no change in DBPCFC symptom score or skin test reactivity for this individual. Unfortunately, the study was prematurely terminated due to a fatal adverse reaction. Overall, the rate of systemic reactions with this rush SCIT protocol was 13.3 %.

In another study of peanut SCIT, 6 subjects received treatment and 6 untreated subjects served as controls. All treated subjects reached a maintenance dose of 0.5 mL of 1:100 wt/vol peanut extract by a rush protocol [11]. After 1 month, those receiving SCIT had improved tolerance as demonstrated by DBPCFC and decreased sensitivity on SPT; the untreated control patients showed no change. Only 3 patients were able to continue on the maintenance dose for 1 year and had continued improvement in tolerance at the post-treatment challenge. The remaining 3 patients required dose reductions for systemic side effects; this resulted in either partial or complete loss of protection. Overall, systemic reactions were common both during the initial rush protocol and with maintenance injections (39 %). Of note, most of the systemic reactions required treatment with epinephrine.

In another case report of successful desensitization for peanut allergy using SCIT, immunologic parameters were assessed [12]. Peanut-specific IgE levels declined from pretreatment levels, but still remained significantly elevated. Increased peanut-specific IgG₄ levels were observed post-treatment. Although these studies demonstrate that SCIT can lead to increased tolerance, unacceptably high rates of adverse systemic reactions are a significant

disadvantage to this method. Furthermore, there are no long-term studies investigating whether the increased tolerance persists after discontinuation of treatment, as it does for aeroallergen immunotherapy.

Oral immunotherapy (OIT)

In recent years, there has been a tremendous interest in oral immunotherapy as a treatment for food allergies, an approach first described over 100 years ago. There is a rapidly expanding body of literature indicating a high rate of efficacy with oral immunotherapy.

Milk allergy

Several early reports demonstrated the beneficial clinical effects of OIT for milk allergy [13–15]. These clinical improvements were associated with decreased specific IgE levels, increased IgG₄ levels and smaller skin test responses to milk.

The first double-blind, placebo-controlled OIT study for food allergy in children was carried out by Skripak et al. [16] for milk allergy. Twenty children were randomized to receive milk OIT or placebo. Dosing occurred in 3 phases: initial build-up under observation to a final dose of 50 mg, daily home doses with weekly dose escalation under observation for 8 weeks to a maximum of 500 mg, and daily home maintenance of 3–4 months. At the post-treatment challenge, all children on active OIT experienced an increased threshold for reaction to milk (median of 5,140 mg compared to median of 40 mg at the baseline DBPCFC). No change was seen in the placebo group. Although there was no significant change in specific IgE levels or skin prick test results, there was a significant increase in milk-specific IgG and IgG₄ in the active treatment group. Notably, the majority of participants experienced reactions during the post-treatment milk challenge, demonstrating that complete protection from allergic reactions due to milk was not achieved. All successfully desensitized participants continued open-label maintenance treatment with milk OIT at home [17]. Although increased tolerance was seen over time, adverse reactions to dosing were common and unpredictable. Of note, one subject had recurrence of clinical reactivity to milk that manifested primarily with gastrointestinal symptoms similar to eosinophilic esophagitis, highlighting the fact that OIT is not without risks.

There are several additional studies in the literature demonstrating the effects and safety of milk OIT using varying patient populations and dosing regimens. Longo et al. [18] reported on their experience with OIT in a highly milk-allergic population (milk-specific IgE > 85 kUA/L and positive DBPCFC to ≤0.8 mL whole milk). Children

were randomized to receive milk OIT or continue strict avoidance. After an initial 10-day inpatient rush protocol, the OIT group continued dose escalation at home to a maximum of 150 mL milk in a single dose. Subsequently, increasing amounts of dairy products were included in the diet. After 1 year, 36 % of the OIT group had unrestricted diets, and more than half (54 %) were able to tolerate limited amounts of milk (ranging from 5 to 150 mL). Of the children not receiving OIT, all had positive DBPCFC at minimal amounts of milk after 1 year. Adverse reactions were common and occurred in all children on OIT. Four children required intramuscular epinephrine several times during the rush phase, and 2 children required treatment in the emergency department during the home dosing phase. Staden et al. [19] reported a case series of 9 high-risk, milk-allergic children who underwent a rush oral immunotherapy protocol with milk. Six participants were able to reach the maximum dose within 3–7 days (5–38 doses; median, 18 doses). Mild side effects were frequent, but no medications were required. Another report evaluated a weekly up-dosing OIT regimen that did not require hospitalization [20]. Ten of the 13 subjects successfully reached the 200 mL maintenance dose over 18 clinic visits. This protocol appears to be safe, as majority of adverse reactions were transient and did not require medication.

As described below, we are investigating whether pretreatment with omalizumab (anti-IgE), which has been shown to reduce allergic symptoms in some children with food allergy and decrease IgE binding on the surface of mast cells, basophils and antigen-presenting cells, will minimize adverse reactions to OIT.

Egg allergy

Fewer studies have focused on egg OIT. One early report included 7 non-anaphylactic egg-allergic children (mean age 4 years) [21]. Subjects received modified rush immunotherapy with build-up to a daily maintenance dose of 300 mg, which was continued for the duration of the study. Four patients who passed a DBPCFC at the end of the 24-month trial underwent a second DBPCFC after 3–4 months of OIT; two patients passed this second DBPCFC, indicating tolerance. A significant increase in egg-specific IgG was seen post-treatment, but no change in egg-specific IgE was observed.

In a randomized trial of egg OIT, 49 received treatment and 35 children continued egg avoidance [14]. Although some improvement in clinical tolerance was seen, it did not reach statistical significance. However, the OIT group did demonstrate decreased skin test responses and egg-specific IgE after the 6-month treatment period.

A recent open-label clinical trial of egg OIT suggested that individualized IgE-based dosing of egg OIT may be

the most effective method to achieve a full therapeutic response [22]. OIT treatment consisted of an initial dose escalation day followed by a build-up to 300 mg. Subsequent dosing increases were determined by egg IgE levels and DBPCFC assessments. Whenever the egg IgE level was $<2 \text{ kU}_A/\text{L}$, OIT was discontinued and tolerance was assessed by DBPCFC immediately following and 1 month of OIT. All 6 who completed the protocol passed an OFC to egg. This was associated with decreased SPT responses, decreased egg- and ovomucoid-specific IgE levels, and increased egg-specific IgG₄.

Peanut allergy

The first studies of peanut OIT emerged in 2009. A case study of 4 peanut-allergic children showed that all had improved tolerance to peanut after peanut OIT to a maintenance dose of 800 mg peanut protein for 6 weeks [23]. In another uncontrolled, open-label study, 39 peanut-allergic children were enrolled (median age, 57.5 months; range, 12–111 months) [24]. Of the 29 who completed all 3 phases of the study and peanut challenges, 93 % completed the 3.9 g peanut protein challenge with no more than mild symptoms after a median of 4.7 months (range 4–22 months) on the maintenance dose of 300 mg peanut protein. These subjects also had smaller SPT and decreased basophil activation by 6 months, and lower peanut-specific IgE was observed after 18 months of therapy. Most symptoms occurring during OIT were transient and responded well to antihistamines. A follow-up report on safety of peanut OIT home dosing found that 3.5 % of home doses were associated with adverse reactions, and epinephrine was required for 3 of the 7 reactions described [25]. Several factors that appeared to increase the likelihood of adverse reactions to OIT included concurrent illness, suboptimally controlled asthma, taking doses on an empty stomach, physical exertion after dosing, and dosing during menses.

Another open-label study of peanut OIT included 23 children with peanut allergy confirmed by DBPCFC [26]. Fourteen completed the study protocol and underwent post-treatment DBPCFC 2 weeks off therapy. Eleven of these subjects were able to tolerate equal or higher amounts of peanut than the maintenance dose. Although no change in peanut-specific IgE was seen during OIT, a significant increase in peanut-specific IgG₄ and decrease in peanut-specific IL-5, IL-4 and IL-2 production by PMBCs was seen after OIT. Regarding safety, adverse reactions were reported in 2.6 % of the over 6,000 home doses given, none of which required epinephrine treatment.

The first double-blind, placebo-controlled study of peanut OIT was recently published by Varshney et al. [27]. Twenty-eight children with non-anaphylactic peanut

allergy (median age, 69 months; range, 28–126) were randomized to receive treatment with peanut OIT or placebo (2:1). OIT was administered with an initial escalation phase, build-up phase and maintenance at 4,000 mg for 1 month. Sixteen of nineteen children randomized to OIT completed 1 year of treatment. All 16 completed the post-treatment OFC of 5,000 mg peanut protein, indicating successful desensitization; only 1 subject required treatment with antihistamine. The median cumulative dose ingested by the 9 subjects on placebo was 280 mg. During OIT, there was no significant change in peanut-specific IgE, but peanut-specific IgG and IgG₄ were increased. Immunologic studies suggested a shift away from a Th2-type profile (lower IL-5 and IL-13 production) and induction of T regulatory cells. OIT was well-tolerated, with only 1.2 % of build-up doses causing allergic reactions. More importantly, no one on OIT required epinephrine during dose escalation or home doses.

These studies demonstrate that OIT is a very promising treatment option for food allergy. Despite this recent surge in published clinical trials data, it is still unclear whether apparent improvements in tolerance are due to true induction of oral tolerance or spontaneous improvement of the food allergy. Furthermore, the immunologic mechanisms underlying OIT have yet to be fully elucidated. Additional trials using placebo controls and mechanistic studies should provide valuable insights.

Oral immunotherapy with heat-denatured proteins

Recent studies from our group have demonstrated that the majority of children with milk and egg allergy are able to tolerate extensively heated milk and egg [28, 29]. The heating process destroys the conformational epitopes that are primarily recognized by children with transient milk and egg allergy [30, 31]. Using a peptide microarray immunoassay, we found that subjects with persistent milk allergy have increased IgE-binding diversity to sequential epitopes compared to those with transient milk allergy and those tolerating extensively heated milk products [32]. Furthermore, we were able to demonstrate for the first time that the allergic phenotype is correlated with antibody affinity. Specifically, milk-allergic patients possess a combination of high- and low-affinity IgE binding to sequential allergenic epitopes, whereas baked-milk-tolerant subjects and those who had outgrown their milk allergy had primarily low-affinity binding to sequential epitopes.

The therapeutic potential for oral immunotherapy with heat-denatured proteins was demonstrated in our longitudinal study of milk-allergic children who are including baked milk products in their diet. We found that many of these children experienced accelerated tolerance induction [33]. Children who were tolerant to baked milk products

and incorporated them into their diets were significantly more likely than the comparison group to become tolerant to unheated milk within 60 months ($P < 0.001$; Fig. 1). Although median milk-specific IgE levels did not change over this time period, a significant increase in median casein-specific IgG₄ levels was seen in the treated group. Addition of the baked milk products into the diets was safe, convenient and well-tolerated.

The effects of heating on allergenicity are variable and food dependent. Although milk and egg allergens can be denatured with heating, high temperatures appear to increase allergenicity of some foods, including peanut and shrimp [34, 35]. Therefore, this form of immunotherapy would not be applicable to all food allergens.

Sublingual immunotherapy

Sublingual immunotherapy (SLIT), which has been demonstrated to be a safe and effective treatment for allergic rhinitis and asthma, is also being evaluated for the treatment for food allergy. It involves the administration of small amounts of allergen under the tongue. The effects of SLIT are believed to be mediated by the uptake of allergen by oral Langerhans cells, which have been shown to have tolerogenic properties [36]. The first case of successful SLIT for the treatment for food allergy was reported in a patient with kiwi anaphylaxis using 1 cm³ of kiwi daily [37]. Efficacy persisted even after the discontinuation of SLIT for 4 months [38]. Clinical improvement was associated with decreased kiwi-specific IgE and increased kiwi-specific IgG₄.

Fig. 1 Development of tolerance: per-protocol (PP) versus comparison groups. The log-rank P value comparing survival between the per-protocol versus comparison groups is less than .001. Subjects in the per protocol group were 3.6 times more likely to have unheated milk tolerance than subjects in the comparison group over the follow-up period (hazard ratio, 3.57; 95 % CI, 1.78–7.16; $P < .001$) adjusted for sex, age at initial visit and baseline milk-specific IgE levels. We present data up to 60 months because beyond 60 months, the CIs were very wide as a result of the large number of censored data (reproduced with authorization from Kim et al. [33])

In a randomized double-blind, placebo-controlled study investigating SLIT for hazelnut allergy, 12 subjects were treated with SLIT for 5 months using the sublingual-discharge (spit-out) technique [39]. Six patients had symptoms consistent with pollen-fruit syndrome to hazelnut, 5 patients had a history of anaphylaxis to hazelnut, and 1 had a non-anaphylactic reaction to hazelnut. The SLIT extract contained Cor a 1 (Bet v 1 homologue) and Cor a 8 (lipid transfer protein). Eleven patients received placebo. Significant increases in threshold of reactivity to hazelnut allergen were seen in the treated group as compared to placebo. Increases in hazelnut-specific IgG₄ and IL-10 post-treatment were also seen in the active group. Treatment was generally well-tolerated; local reactions occurred in 7.4 % and consisted mostly of oral pruritus. The systemic reaction rate was low (0.2 %), occurring only during the build-up phase. For 7 actively treated subjects, SLIT was discontinued for 4 months, resumed at the maintenance dose (without a build-up phase) and then continued for 1 year [40]. Significant increases in hazelnut doses were tolerated at the post-treatment DBPCFC, demonstrating the beneficial effect of SLIT even after treatment interruption.

Recently, SLIT for peanut allergy has been investigated in a double-blind, placebo-controlled study [41]. After 12 months of therapy (6-month build-up and 6-month maintenance), the active treatment group tolerated approximately 20 times more peanut protein during food challenge than the placebo group (median, 1,710 vs. 85 mg, $P = 0.011$) indicating clinical desensitization. This improved tolerance was associated with decreased skin prick test wheals, lower peanut-specific IgE levels, increased peanut-specific IgG₄ levels, and lower percentage of CD63⁺ basophils when stimulated with crude peanut extract in the active group at 12 months. This treatment was well-tolerated; <0.3 % of doses required treatment with either antihistamines or albuterol.

An ongoing double-blind, placebo-controlled randomized trial of peanut SLIT is being conducted through the Consortium of Food Allergy Research, which is centered at Mount Sinai (ClinicalTrials.gov identifier NCT00580606). This larger, multicenter study should provide further insight into mechanistic effects, efficacy and safety of SLIT.

Epicutaneous IT (EPIT)

Another route for administering immunotherapy that is being investigated for food allergies is epicutaneous immunotherapy (EPIT). This involves repeated application of allergens to intact skin and a new delivery system that has been tested in a pilot study involving milk-allergic children [42]. EPIT was generally well tolerated with most

adverse events involving local irritation that was treated with topical medications, and these adverse events did not interrupt therapy. Although treatment duration was only 3 months and no statistically significant improvements in milk tolerance or milk-specific IgE were seen, these study results suggest that further exploration of EPIT is warranted.

A large multicenter, double-blind placebo-controlled trial of peanut EPIT is being carried out by our Consortium of Food Allergy Research (ClinicalTrials.gov identifier: NCT01170286), which should provide evidence as to the utility of this approach.

Immunotherapy with modified recombinant vaccines

Modified recombinant food proteins have been investigated for use in immunotherapy to reduce the incidence of adverse effects. These recombinant proteins retain the ability to stimulate T-cell responses, but have greatly reduced IgE-binding capacity as compared to wild-type peanut protein [43, 44]. These can be combined with bacterial adjuvants to enhance the Th1 and Treg skewing effects. We showed that heat-killed *E. coli* (HKE) producing engineered recombinant peanut proteins have protective effects in a murine model of peanut anaphylaxis [45]. Mice treated with HKE-EMP123 demonstrated reduced symptom scores during peanut challenge as compared to the placebo-treated group. This protection persisted up to 10 weeks post-treatment in mice treated with medium- and high-dose HKE-MP123. The high-dose-treated group demonstrated the most significant decrease in IgE levels and decreased production of IL-4, IL-5, IL-13 and IL-10, and increased IFN- γ and TGF- β production by peanut protein-stimulated splenocytes in vitro. The proposed protective mechanisms involve generation of Th1 cytokines and/or upregulation of T regulatory cells suppressing Th2 cell activation and mast cell/basophil mediator release on re-exposure to antigen [46, 47]. A phase 1, open-label trial of EMP-123 (a rectally administered modified recombinant Ara h 1, Ara h 2, Ara h 3 encapsulated in heat/phenol-killed *E. coli*) was recently completed at Mount Sinai and Johns Hopkins [48]. Five healthy adults received 4 weekly escalating doses with no significant adverse effects. Ten peanut-allergic adults were treated with 10 weekly escalating doses followed by 3 biweekly doses. Of these subjects, 5 discontinued the protocol due to allergic reactions, 1 had mild rectal symptoms, and 4 tolerated treatment with no adverse reactions. Of note, the 5 subjects experiencing allergic reactions had significantly higher peanut-specific IgE levels. Post-treatment peanut skin test results were significantly reduced from baseline ($P = 0.02$); however, no significant changes were detected for total IgE, peanut-specific IgE, peanut-specific IgG₄ or

peanut-induced basophil activation (CD63+ and CD203+).

Additional strategies in the preclinical stage

The premise underlying the use of food allergen peptide immunotherapy involves the use of short peptides that have absent or reduced IgE binding, but intact T-cell binding as a means to induce tolerance in a safer manner. A preliminary *in vitro* study using pepsin-digested peanut peptides showed induction of IFN- γ (Th1 cytokine) from peripheral blood mononuclear cells in a concentration-dependent manner [49]. In a murine model of peanut allergy, we were able to demonstrate that mice receiving peptide immunotherapy to the major peanut protein, Ara h 2, prior to allergen challenge had milder allergic reactions as compared to sham-treated mice, which exhibited severe anaphylactic reactions [50]. Encouraging results were also seen in a murine model of egg allergy, in which oral immunotherapy with T-cell epitope peptides from ovomucoid led to less frequent clinical symptoms upon oral challenge with ovomucoid [51]. This was associated with lower specific IgE levels, lower IL-4 production and increased T regulatory cell percentage.

Development of peptide immunotherapy for peanut allergy in humans has begun, using Ara h 2. T-cell epitopes have been mapped using synthetic overlapping Ara h 2 peptides in T-cell proliferation assays from peripheral blood mononuclear cells [43] and peanut-specific CD4+ T-cell lines [52] from peanut-allergic individuals. Recently, our laboratory identified 4 regions in Ara h 2 that significantly induced PBMC proliferation; 3 of these match previously identified areas [43, 52]; however, the fourth area was identified for the first time, and notably, a part of this sequence corresponds to the Ara h 2 signal peptide [manuscript submitted]. In addition, these immunodominant regions were also identified using MHC-class-II-based T-cell epitope prediction algorithms for HLA-DR and HLA-DQ loci, indicating that the *in silico* approach may be an additional tool to facilitate the identification of candidate peptides for immunotherapy.

Allergen gene immunization to immunomodulate allergen-induced allergic responses has been explored by Roy et al. [53]. DNA nanoparticles containing the gene for Ara h 2 were synthesized by complexing plasmid DNA with chitosan. Mice were immunized with chitosan–pArah2 nanoparticles, ‘naked’ pArah2 or not immunized, sensitized with oral and intraperitoneal doses of crude peanut extract, and then challenged intraperitoneally with recombinant Ara h 2 protein. Mice orally immunized with these nanoparticles demonstrated less severe and delayed anaphylactic responses following peanut challenge compared to mice treated with ‘naked’ DNA or unimmunized mice.

Decreased IgE levels, lower plasma histamine and less vascular leakage were also seen. It is important to note that the type of immune responses to ‘naked’ DNA immunization in mice is strain dependent [54]. We found that C3H/HeSn mice immunized with plasmid Ara h2 DNA exhibited signs of anaphylaxis after peanut protein challenge, whereas immunized AKR or BALB/c mice did not exhibit any signs of anaphylaxis post-challenge, suggesting that variations in responses may be likely in humans as well.

Yet another approach utilizes the knowledge that sugar-modified antigens have been shown to target tolerogenic dendritic cells. In a murine model of food allergy, oral administration of bovine serum albumin (BSA) coupled with mannoside led to diminished anaphylactic responses after challenge with unmodified BSA; this was associated with significantly decreased plasma histamine levels and vascular permeability [55]. The mechanism of this protection was shown to be due to activation of the C-type lectin receptor (SIGNR-1 or CD202b), which has a role in induction of T regulatory type 1 cells (Tr1).

Allergen-non-specific therapies

Several therapeutic strategies are being investigated to downregulate the overall allergic response in food-allergic individuals (by lowering IgE or promoting Th1 immune responses). These approaches would be particularly beneficial to those with multiple food allergies (Table 2). Some of these treatments may change the threshold for allergic reactions (i.e., anti-IgE), whereas others may have more persistent effects (i.e., Chinese herbal medicine).

Anti-IgE therapy

Recombinant monoclonal humanized anti-IgE is currently used for the treatment for environmental allergies associated with asthma. Its effects include reduction in circulating free IgE, inhibition of the early- and late-phase allergic response, suppression of inflammation and improved control of allergic symptoms [56]. In an early multicenter trial with the anti-IgE antibody TNX-901, we and others demonstrated that peanut-allergic patients who received the anti-IgE treatment (450 mg monthly for 4 months) had a significant decrease in symptoms with peanut challenge as compared to the placebo group [57]. Furthermore, the median threshold of sensitivity to peanut increased from 178 mg peanut protein (the equivalent to one peanut) to almost 9 peanuts (2.8 g). Although 25 % of patients were able to tolerate over 20 peanuts following therapy, another 25 % failed to develop any improvement in tolerance to peanut following treatment. Recently, we led an effort to

investigate another anti-IgE preparation, omalizumab (Xolair[®], Genentech), as a putative therapy for peanut allergy in a randomized, double-blind, parallel-group, placebo-controlled study. Although the study planned to randomize 150 subjects, the trial was suspended early due to the recommendation of the Data Safety Monitoring Committee because of the concerns related to the severity of 2 anaphylactic reactions that occurred during the screening oral food challenges at two study sites prior to the administration of any study drug [58]. Prior to suspension, 14 subjects completed the post-therapy (24-week) oral food challenge. Based on the limited data, there appeared to be a greater shift in peanut tolerability in subjects treated with omalizumab as compared to placebo.

Use of anti-IgE in conjunction with oral immunotherapy to milk protein is currently being investigated at our center as a method to allow higher and more rapid dosing of immunotherapy in a safer manner (Clinicaltrials.gov identifier NCT01157117). Reducing free IgE is hypothesized to decrease adverse reactions by eliminating or markedly decreasing milk protein-induced mast cell and basophil activation during oral immunotherapy and markedly reducing facilitated allergen presentation by IgE-bearing antigen-presenting cells, which promotes Th2 induction. Nadeau et al. [59] recently carried out a pilot phase I study in 11 milk-allergic children. Nine of these subjects achieved the primary objective or reaching an OIT dose of 2,000 mg/day within 7–11 weeks. All 9 of these subjects passed a DBPCFC and an open challenge and continued with daily milk ingestion (>8,000 mg/day).

Traditional Chinese medicine (TCM)

Traditional Chinese medicine (TCM) has been used for thousands of years to treat a variety of diseases. Recently, TCM has attracted more interest in Western countries as a source of alternative or complementary therapy for a variety of diseases, including allergies and asthma. Our group developed a formula of 9 herbs designated food allergy herbal formula (FAHF-2) to treat food allergies based on TCM [60]. This formula has been shown to protect peanut-sensitized mice from anaphylaxis after peanut challenge. These clinical effects were associated with decreased peanut-specific IgE levels and Th2 cytokine production (IL-4, IL-5 and IL-13). Furthermore, the protective effects of FAHF-2 were demonstrated to last up to 6 months post-therapy, which represents about 25 % of the life span of the mouse [61]. These results were initially based on mice given the FAHF-2 during peanut sensitization, but additional studies have demonstrated that FAHF-2 can also induce tolerance to peanut in mice with established peanut allergy [62].

Mechanistic studies demonstrated that the beneficial effects of FAHF-2 were mediated largely by elevated

CD8(+) T-cell IFN-gamma production [63]. Mice that received CD8(+) T-cell-depleting antibodies or IFN-gamma-neutralizing antibodies had smaller decreases in allergen-specific IgE and the protective effects of FAHF-2 were not complete or durable. Further studies suggest a beneficial effect of FAHF-2 on mast cells and basophils as well [64]. Mice treated with FAHF-2 had a reduction in peripheral blood basophils and decreased number and FcεRI expression of peritoneal mast cells 4 weeks post-therapy. Using a murine mast cell line (MC/9), our group showed that FAHF-2 treatment results in a significant reduction in IgE-induced FcεRI expression, FcεRI γ mRNA subunit expression, proliferation and histamine release upon challenge. These effects likely contribute to the durability of FAHF-2 protection against peanut anaphylaxis.

In vitro studies using purified human peripheral blood mononuclear cells obtained from peanut-allergic individuals show that in the presence of FAHF-2, there was decreased antigen-dependent T-cell proliferation stimulation. There was also a dose-dependent decrease in Th2 cytokine production (IL-5 and IL-13) and increase in IFN-gamma production, indicating that FAHF-2 specifically inhibits the Th2 response [65].

A randomized, double-blind, placebo-controlled, dose escalation phase I trial was conducted which demonstrated that FAHF-2 was safe and well tolerated in food-allergic individuals [66]. An open-label, extended phase I study was then completed to assess long-term safety [67]. Fourteen subjects took 6 tablets three times a day for 6 months. No significant differences in laboratory parameters, pulmonary function tests, or electrocardiographic findings were seen before and after treatment. Although not an end point of the study, immunologic parameters were evaluated and a significant reduction in basophil CD63 expression was observed in response to ex vivo allergen stimulation after 6 months of FAHF-2 treatment. A trend in decreased circulating eosinophil and basophil numbers was also seen.

Currently, a multicenter, double-blind, placebo-controlled phase 2 study is underway (Clinicaltrials.gov identifier: NCT00602160).

Anti-IL5 therapy

Since Th2 cytokines play important roles in the development of the allergic response, strategies to block their actions have been investigated as potential therapeutic approaches for various allergic disorders. Eosinophilic esophagitis (EoE) is an inflammatory disorder characterized by high numbers of intraepithelial eosinophils in the esophagus [68]. Although the etiology of EoE is still unclear, the majority of patients have IgE-mediated food and aeroallergen sensitization. Current therapies include

elemental or hypoallergenic diets and topical corticosteroids; however, compliance with an elemental diet is very difficult and corticosteroids are associated with local and systemic side effects. On the basis of the murine models of EoE and analysis of human esophageal tissue indicating the presence of T cells and mast cells along with eosinophils, EoE appears to be mediated by a Th2-based inflammatory process [69, 70]. Since IL-5 is a major Th2 cytokine and is a regulator of eosinophil function and survival, anti-IL-5 (mepolizumab) has been investigated for the treatment for EoE. In an open-label phase I/II study of anti-IL-5 in 4 adults with EoE, 3 infusions of anti-IL-5 (750 mg intravenously monthly) resulted in marked decreases in peripheral and esophageal eosinophilia and symptomatic improvement [71]. Another trial in adults also demonstrated significant reduction in intraepithelial eosinophils, but minimal clinical effects [72]. Our group participated in the recently published international, multicenter, double-blind, randomized, stratified, parallel-group study of mepolizumab in children with EoE [73]. Subjects received an infusion of mepolizumab every 4 weeks for 3 months (0.55, 2.5, or 10 mg/kg). Although a complete response (peak esophageal intraepithelial eosinophil count of <5 per hpf) was seen in only 8.8 %, nearly 90 % had a reduction in mean eosinophil counts to <20 per hpf. This reduction was associated with improved endoscopic findings, but a limited effect on clinical symptoms. Additional larger-scale studies are needed to establish the potential role of anti-IL5 in the treatment for EoE.

Toll-like receptors

Since signaling through Toll-like receptors (TLR) leads to strong mucosal and systemic Th1-type immune responses, TLR agonists have been investigated as potential treatments for food allergy. Oral administration of a synthetic agonist of TLR9 to treat peanut allergy was investigated by Zhu et al. [74], using a murine model. They demonstrated that treatment with the TLR9 agonist protected the mice from anaphylaxis during peanut challenge regardless of whether it was administered during peanut sensitization or after peanut allergy was established. This was associated with lower peanut-specific IgE and higher specific IgG2a in serum as well as with decreased IL-5 and IL-13 production by splenocytes. Our group has performed preliminary studies with peanut proteins linked with CpG, a TLR9 antagonist, in a murine model of peanut-induced anaphylaxis [75]. Mice were immunized with either ISS-linked Ara h 2 (a major peanut protein) or ISS-linked Amb a 1 prior to sensitization with peanut. Mice treated with ISS-linked Ara h 2 had lower symptom scores and lower plasma histamine levels following challenge with Ara h 2 compared to mice treated with ISS-linked Amb a 1. Although significantly higher Ara h

2-specific IgG2a levels in the ISS-linked Ara h 2-treated mice were observed, there was no significant difference in specific IgE or IgG₁ levels between the two groups. Another example of employing TLR activation to promote Th1 skewing is the use of *E. coli* in EMP-123 (engineered recombinant peanut proteins discussed above) [48]. At this time, we are exploring the use of nanoparticles containing peanut protein and LPS and/or CpG in a murine model of peanut allergy.

This type of approach has also been investigated using a TLR5 agonist. Schulke et al. [76] investigated the effect of a recombinant fusion protein consisting of *Listeria monocytogenes*-derived flagellin A (flaA) with OVA in a murine model of intestinal allergy. Vaccination with flaA:OVA led to reduction in clinical symptoms after challenge with egg white, suppression of OVA-specific IgE, and reduction of T-cell activation.

Probiotics

Probiotics have been actively investigated for the treatment for atopic dermatitis, asthma and allergic rhinitis with variable results [77, 78]. Given that commensal bacteria have been shown to play an important role in immune modulation and the development of tolerogenic immune responses [79], use of probiotics in the treatment for food allergy has been explored. Murine studies have suggested a beneficial effect of probiotics. Using a murine model of shrimp allergy, Schiavi et al. demonstrated that oral treatment with a probiotic mixture VSL#3 (VSL Pharmaceuticals, Fort Lauderdale, FL) protected mice from shrimp tropomyosin-induced anaphylaxis [80]. A significant decrease in IL-4, IL-5 and IL-13 as well as an increase in IFN-gamma, IL-10, TGF-beta, and IL-27 was observed in the jejunum.

Human trials have not shown similar benefits. In a double-blinded, placebo-controlled study, 230 infants with atopic dermatitis were randomized to receive probiotics (single strain of *Lactobacillus rhamnosus* GG (ATCC53103) 5×10^9 colony-forming units (CFU) or in combination with *L. rhamnosus* (LC705) 5×10^9 CFU, Bifidobacterium breve Bbi99 2×10^8 CFU, and Propionibacterium freudenreichii ssp. shermanii JS 2×10^9 CFU) or placebo for 4 weeks [81]. There was no significant difference in skin symptoms between those who received probiotics as compared to placebo. In a subgroup of children who had concomitant milk allergy (52 %), no difference was noted as well. In another double-blind, placebo-controlled trial in infants with milk allergy using a different combination of probiotics (*Lactobacillus casei* CRL-431 and Bifidobacterium lactis Bb-12), no difference was seen in the rate of acquisition of milk tolerance between the groups after 12 months of treatment [82].

Although these few studies do not show benefit, it is unclear whether the use of different strains or combinations as well as variations in dosing, delivery method, and duration of treatment could lead to improved clinical results.

Helminths

Allergic disorders are characterized by Th2 skewed immune responses. However, parasitic helminth infections, which are also potent inducers of Th2 immune responses, are associated with lower rates of allergy [83, 84]. In a murine model of peanut allergy, Bashir et al. [85] found that helminth-infected mice have diminished allergic symptoms and lower peanut-specific IgE levels. Although an intriguing concept, a recent clinical trial using oral administration of *Trichuris suis* ova for allergic rhinitis in humans did not show any clinical benefits [86]. Adverse events included significant gastrointestinal complaints, particularly in the first 2 months of treatment [87]. Similar disappointing results were seen in a study exploring cutaneous application of *Necator americanus* for the treatment for asthma [88]. In addition to the lack of clinical improvement, no change in skin test reactions to environmental allergens was observed at 16 weeks post-therapy. Adverse reactions included localized skin itching and redness as well as mild gastrointestinal complaints. A clinical trial using *Trichuris suis* ova is currently underway for nut allergies (ClinicalTrials.gov identifier: NCT01070498).

Conclusion

Food allergy is an increasingly common problem, with wide-ranging affects on daily life. Given the lack of treatment options at this time, the development of therapies for food allergy that are safe and effective is a high priority. We have described several promising approaches that are currently being investigated in our institution as well as in others. These strategies, either alone or in combination, will hopefully provide long-term treatment options and potentially a cure for food allergy. Moreover, information from these studies will further advance our understanding of the underlying mechanisms of tolerance.

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