



Enhancing the Safety and Efficacy of Food Allergy Immunotherapy: a Review of Adjunctive Therapies

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Abstract

Food allergy is a potentially life-threatening condition with no approved curative therapy. A number of food allergen immunotherapies are being investigated in phase II/III trials; however, these are limited in their ability to restore immune tolerance to food allergens and often result in high rates of allergic side effects, sometimes involving anaphylaxis, that may curtail their impact. A variety of adjunctive therapies have been developed in order to enhance the efficacy and/or improve the safety of food allergen immunotherapy through either shifting the immune response from a Th2 polarized response to a Th1 and regulatory T cell dominated response or by blocking downstream effects of the allergic inflammatory response by targeting IgE or mast cell mediators. Upstream therapies that shift towards a Th1/Treg response include toll-like receptor (TLR) 4 agonists (e.g., MPL and GLA), TLR9 agonists (CpG oligonucleotides), nanoparticles encapsulating peanut allergen (with and without adjuvants, such as CpG or rapamycin), Chinese herbal medicine (food allergy herbal formula (FAHF-2)), probiotics, and interferon-gamma. In contrast, anti-IgE therapies such as omalizumab, anti-histamines like ketotifen, and leukotriene receptor antagonists all target the downstream allergic response. Anti-IgE-based therapies appear to be furthest along with probiotics, Chinese herbal medicines, and TLR-4 agonists currently in early phase clinical trials. Meanwhile, nanoparticles represent an innovative delivery vehicle for immunotherapy that could improve both efficacy and decrease allergic side effects. Furthermore, other biologic therapies directed towards the allergic immune response are on the horizon. A number of factors will need to be evaluated in comparing these treatments, including ability to decrease allergic adverse events, safety of the adjunctive therapies themselves, effect on long-term sustained unresponsiveness, and cost. Further phenotyping of food allergy patients may be necessary to determine which ones respond best to each therapy. However, with so many promising adjunctive therapies, it appears likely that clinicians will have a variety of options to optimize the administration of food allergen immunotherapy. We provided a review of these methods, their influence on allergic adverse events, and utility in improving the immunomodulatory effects of food allergen immunotherapy.

Keywords Food allergy · Oral immunotherapy · Immunotherapy · Anti-IgE · Omalizumab · Nanoparticles · Toll-like receptor agonists

Abbreviations

IgE	Immunoglobulin E	MPL	Monophosphoryl lipid A
TLR	Toll-like receptor	GLA	Glucopyranosyl lipid A
Th	T helper	OVA	Ovalbumin
CpG	Cytosine-phosphate-guanine	mTOR	Mammalian target of rapamycin
ISS	Immunostimulatory sequence	FAHF-2	Food allergy herbal formula 2
		LTRA	Leukotriene receptor antagonists
		DBPCFC	Double-blind, placebo-controlled food challenge

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Introduction

Food allergy is a potentially life-threatening condition affecting approximately 3–8% of US children [1–3]. Given the lack of approved curative therapy, management is limited to

allergen avoidance and supportive measures if an allergic reaction occurs [4, 5]. A major focus of current clinical research is the development of disease-modifying treatments that can provide clinical protection against an accidental exposure. Many of the treatments involve exposing the allergic individual to daily doses of allergen in order to transiently raise the reaction eliciting dose of allergen. These protocols generally involve a short initial escalation day, followed by a slow build-up period during which doses are increased over weeks to months, and then a final maintenance period of stable dosing. These treatments have been evaluated on the basis of three main clinical outcomes: (1) desensitization, (2) sustained unresponsiveness, and (3) safety. Desensitization describes a state where regular exposure to the allergen raises the dose of allergen required to trigger an allergic reaction; this state is transient and requires continued administration of the allergen to maintain protection. Sustained unresponsiveness refers to a more durable level of protection where food allergic patients are able to consume the allergen even after immunotherapy has been discontinued for weeks to months. However, because the duration of even this protection has been shown to wane for some over extended time off immunotherapy, sustained unresponsiveness is distinct from normal tolerance, as experienced by non-allergic individuals. Regarding safety, many of these treatments are characterized by persistent allergic reactions, where daily exposure to the allergen, depending upon the dose and route, frequently triggers allergic reactions, ranging from mild mouth itch or rash to severe anaphylaxis requiring epinephrine.

Oral immunotherapy (OIT), now being evaluated in phase III trials ([ClinicalTrials.gov](https://clinicaltrials.gov); NCT02635776), is one of the most investigated of these therapeutic measures, and involves ingesting gradually increasing amounts of allergen (increasing up to a maintenance dose of hundreds or thousands of milligrams per day) [6, 7]. OIT for peanut allergy has been shown to successfully desensitize a majority of peanut-allergic children, which has generated excitement about OIT for peanut allergy, but significant concerns remain regarding its safety [6, 8, 9]. Most children experience mild allergic adverse events, generally involving mouth itch or abdominal pain. However, a significant proportion experiences systemic reactions or anaphylaxis (potentially as high as 40%) triggered by their daily oral immunotherapy, many of which are severe enough to require epinephrine, with some estimates as high as 12% [8–11]. Sublingual immunotherapy, similar to OIT, involves the administration of increasing doses of allergen but is given sublingually at much lower doses, on the order of 2–4 mg a day [12, 13]. Accordingly, patients on sublingual therapy have a lower level of desensitization to allergen but also experience much fewer severe allergic reactions [14]. Finally, epicutaneous therapy involves the application of a patch to deliver allergen via the skin, reaching doses of 250 µg a day, with the same trade-off of decreasing allergen protection but vastly improved safety profiles consisting primarily of local mild reactions at the site of patch application [15].

Generally, the majority of patients are able to achieve desensitization on these therapies; while definitions of desensitization and sustained unresponsiveness may vary between the studies, rates of desensitization range from 36 to 100% for OIT, 10–70% for SLIT, and 46–48% for EPIT and rates of sustained unresponsiveness range from 27 to 92% for OIT and 10–100% for SLIT [16]. However, as the level of protection increases and patients are able to ingest larger doses of allergen without symptoms, the risk and severity of unexpected allergic reactions also increases.

Given these safety concerns, food allergen immunotherapeutic research has branched out into alternative methods to reduce this risk, including (1) biologics (e.g., anti-IgE monoclonal antibodies), (2) novel delivery vehicles (e.g., nanoparticles with and without adjuvants as well as recombinant and peptide immunotherapy, covered in more detail in Cook et al. [17]), (3) adjuvants designed to target toll-like receptor pathways (e.g., TLR-9 and TLR-4 agonists, probiotics), and (4) other innovative alternatives (e.g., Chinese Herbal medicines, interferon-gamma, ketotifen, and leukotriene receptor antagonists) (Table 1). These modified therapies involve strategies that are intrinsically non-specific to the allergen, but when combined with allergen-specific immunotherapy, are thought to improve or hasten the efficacy of the immune response, reduce allergic adverse events, or both.

Immunologic Mechanisms of Food Allergy

Development of Tolerance in Humans

The ultimate goal of food allergen immunotherapy is to restore the state of clinical tolerance absent in food-allergic individuals. This tolerance is often characterized as an immune-mediated non-responsiveness to food allergen [19, 20]; however, our understanding of the normal physiological immune response is limited. Food allergic individuals are most obviously distinguished from healthy individuals by their production of high-affinity allergen-specific IgE, though clinically tolerant people sometimes have detectable levels as well, underscoring the importance of other differences. It is also known that allergic people have increased numbers of highly type 2 helper (Th2)-polarized effector T cells, characterized in part by their expression of cytokines involved in multiple aspects of allergic inflammation, including IL-4, IL-5, IL-9, and IL-13, a phenotype that is essentially absent in healthy individuals and decreased following immunotherapy [21]. Regulatory T cells (Tregs) have also been implicated in food allergy pathogenesis based on several lines of evidence reviewed elsewhere.

Once sensitized, ingestion of the allergen will trigger the allergen-specific IgE, activating mast cells and basophils to release a variety of inflammatory mediators like histamine and leukotrienes, resulting in an allergic reaction. The adjunctive therapies below attempt to modulate this food allergic

Table 1 A summary of adjunctive therapies for food allergen immunotherapy

Adjunct/adjuvant (concomitant allergen)	Description/mechanism	Pros	Cons	Research status
Anti-IgE (peanut, cow's milk, egg, multi-food OIT)	Blocks the receptor binding site on IgE leading to lower free IgE and downregulation of IgE receptors	Lowers rates of allergic reactions from OIT, and allows for faster dose escalation, with some utility shown for multiple food allergen immunotherapy	No effect on likelihood of developing sustained unresponsiveness. Expensive	Phase II clinical trials
TLR-9 agonists: CpG (peanut, ragweed)	Free CpG oligonucleotides or CpG bound to nanoparticles bind TLR-9 and stimulate Th1 and Treg responses	Decreases the severity of anaphylaxis, increases IFN- γ , and peanut-specific IgG2a antibodies in therapeutic mouse models of food allergy	Some concerns that Th1 response will dominate any T regulatory effects and may have autoimmune potential	Preclinical
TLR-4 agonists: MPL/GLA (peanut, grass, ragweed, tree pollen, and dust mite)	Attenuated or synthetic lipopolysaccharides that bind TLR4 and stimulate Th1 and Treg responses	In aeroallergen immunotherapy, allows for faster buildup and higher response rates. In peanut immunotherapy, lower anaphylaxis scores in mouse models	Some concerns that Th1 response will dominate any T regulatory effects and may have autoimmune potential	Phase I/II trials in allergic rhinitis, and phase I in peanut OIT
Nanoparticles (egg, peanut)	Encapsulates allergen, allowing for improved antigen uptake by antigen presenting cells	Decreased anaphylaxis scores and adverse reactions in mice compared to standard OIT. Allows for incorporation of other adjuvants, potentially with better efficacy (e.g., CpG, rapamycin)	Concerns about release profile and potential for burst release of large amounts of allergen at once	Preclinical in food allergy. Phase I/II clinical trials in cancer
Peptide and recombinant vaccine immunotherapy (apple, cow's milk, egg, fish, peach, peanut)	Uses peptides or modified allergens incapable of cross-linking IgE and activating basophils and mast cells. Stimulates Th1 and Treg responses	Potentially safer given reduced allergenicity. Protection against anaphylaxis in murine models	Requires extensive mapping of T cell epitopes. Phase I trial for <i>Escherichia coli</i> encapsulated recombinant peanut proteins associated with increased adverse events and anaphylaxis [18]	Preclinical and phase I in food allergy. Phase I/II clinical trials for aeroallergens
Chinese herbal medicine (multi-food OIT)	Decreases Th2 responses, as well as IgE-mediated mast cell proliferation and degranulation	Safe, well-tolerated. Decreased adverse reactions and longer post-treatment protection in murine models	Number of tablets increases difficulty of medication adherence. Phase II trial of FAHF-2 monotherapy showed no change in allergen threshold, though possibly due to poor adherence	Phase II trials (B-FAHF-2 with omalizumab and multi-allergen OIT)
Probiotics (peanut)	Stimulates Th1 and T regulatory responses	Potentially increased rates of sustained unresponsiveness	Studies limited by lack of comparison with OIT only arm, differences in protocols for evaluating sustained unresponsiveness. Require more data on strain-specific effects	Phase II trials
Interferon-gamma (egg, cow's milk, wheat)	Main mediator of Th1 response and suppresses Th2 differentiation	Allows for rapid dose escalation without modified rush OIT	Yet to be evaluated against a more traditional buildup period. Unknown if Th1 response will dominate any T regulatory effects	Pilot clinical studies
Ketotifen (peanut)	Anti-histamine and mast cell stabilizer, thereby reducing the severity of allergic reactions	Trends of lower adverse event rates in ketotifen arm, though limited by small sample sizes	Needs replication in larger cohort to evaluate efficacy. Effects on sustained unresponsiveness unknown	Pilot clinical studies
Leukotriene receptor antagonists (egg, cow's milk, wheat)	Inhibits binding of leukotrienes to their receptor, thereby reducing the severity of allergic reactions	Reduced allergic symptoms and allowed progression of OIT in patients who failed OIT alone	Needs replication in larger cohort to evaluate efficacy. Effects on sustained unresponsiveness unknown	Pilot clinical studies

response in two major ways: (1) shifting the immune response towards Th1 pathways and the development of Treg cells and (2) inhibiting the downstream effectors of the Th2-driven allergic response such as IgE, histamine, and leukotrienes (Fig. 1).

Mouse Models of Food Allergy

Many of the adjuvants below have only been investigated in preclinical trials using mouse models of food allergy. To study food allergy in mice, mice are exposed to an allergen, like peanut or ovalbumin (OVA; egg white protein) along with an adjuvant, like cholera toxin (Fig. 2) [22]. This exposure can occur through a variety of routes, including oral, intraperitoneal, and cutaneous. Mice are then challenged to the allergen and monitored for signs of anaphylaxis, such as hypothermia, and often given anaphylaxis scores. Mice can then be given immunotherapy and studied for changes in severity of anaphylaxis. Other immunological measures that are followed include plasma histamine levels, IgE levels, IgG1 (a murine Th2 marker), and IgG2a (a murine Th1 marker).

Murine food allergy research generally takes two approaches, studying prevention models or treatment models. In prevention models, the therapy of interest is administered prior to the sensitization process described above, and then, differences in the level of sensitization, as measured by anaphylaxis scores, are compared to mice that received placebo therapy. In treatment models, mice are sensitized, and then, the therapy of interest is administered, often with concurrent immunotherapy. Mice are then challenged to the food to determine the effect of the therapy of interest with the immunotherapy. In general, the treatment murine models are more clinically applicable as most therapies would likely be targeted to patients with preexisting food allergy.

Biologics: Anti-IgE Therapy

Early Uses of Anti-IgE Monoclonal Antibodies to Reduce Allergen Threshold

Much of the research combining biologics with OIT has focused on anti-IgE monoclonal antibodies, which block the receptor binding site on IgE. This interaction results in decreased levels of free IgE, and corresponding downregulation of IgE receptors on mast cells and basophils [23, 24]. While there are many other new biologic therapies addressing targets such as anti-IL4R α , anti-IL-5, anti-IL-5R α , and anti-IL-13, among others, most of these products have not yet been studied in conjunction with food allergy or OIT, though plans to evaluate dupilumab, the anti-IL4R α monoclonal antibody, as an adjunct to OIT were recently announced.

The first application of the anti-IgE antibody in the setting of food allergy was demonstrated in 2003 using a product that

has now been discontinued, TNX-901 [25]. Leung et al. conducted a double-blind randomized placebo-controlled trial in 84 peanut allergic patients, where they administered TNX-901 and then assessed changes in their threshold for peanut allergen. Participants underwent a baseline peanut oral food challenge to assess their threshold of sensitivity to peanut allergen, and then were randomized to 4 weeks of placebo or one of three different doses of TNX-901. Repeat oral food challenge was conducted 2–4 weeks after the final dose to assess changes in allergen threshold. Mean threshold of peanut increased with increasing doses of omalizumab, though only the highest dose was associated with a statistically significant improvement in allergen threshold, with mean ingestion of 2805 mg of peanut (approximately nine peanuts) compared to the mean ingestion of 178 mg (about half a peanut) in the placebo group. In the highest-dosing group, 76% of patients had achieved an increase in allergen threshold, suggesting that the remaining patients might need an even higher dose of TNX-901 to respond or may not respond at all. Overall, TNX-901 was well tolerated compared to placebo with similar incidences of adverse events.

Though TNX-901 was not brought to market, another anti-IgE monoclonal antibody, omalizumab, was approved for asthma and chronic idiopathic urticaria. A randomized, double-blind, placebo-controlled phase II trial by Sampson et al. was initiated to study the efficacy of omalizumab in reducing peanut allergen threshold [26]. The study intended to enroll 150 participants, but due to two severe anaphylactic reactions during preliminary screening peanut challenges prior to any drug administration, the study was terminated early. At that time, 26 subjects were randomized, with 17 receiving omalizumab. Preliminary results showed a trend towards greater doses of peanut tolerated in the active treatment group, but these were not statistically significant ($p = 0.054$). These results are limited by underpowering due to premature termination of the study. Future trials evaluating the efficacy of omalizumab to increase allergen threshold will be necessary and particularly useful for individuals with multiple allergies that may complicate the administration of OIT.

Pilot Studies of Omalizumab as an Adjunct to OIT

In 2011, the first pilot, open-label, single-arm, phase I study by Nadeau et al. reported the use of anti-IgE therapy combined with OIT with the goal of accelerating the desensitization buildup period and minimizing side effects [27]. This trial studied 11 children with IgE-mediated cow's milk allergy, as diagnosed by history and milk-specific IgE and skin testing. The participants were pretreated with omalizumab (dosed every 2–4 weeks) for 9 weeks, followed by a 7–11-week buildup phase (in contrast with usual buildup phases of 6–12 months) with concurrent omalizumab dosing and a goal maintenance dose of 2000 mg of milk. Ten subjects remained in the study

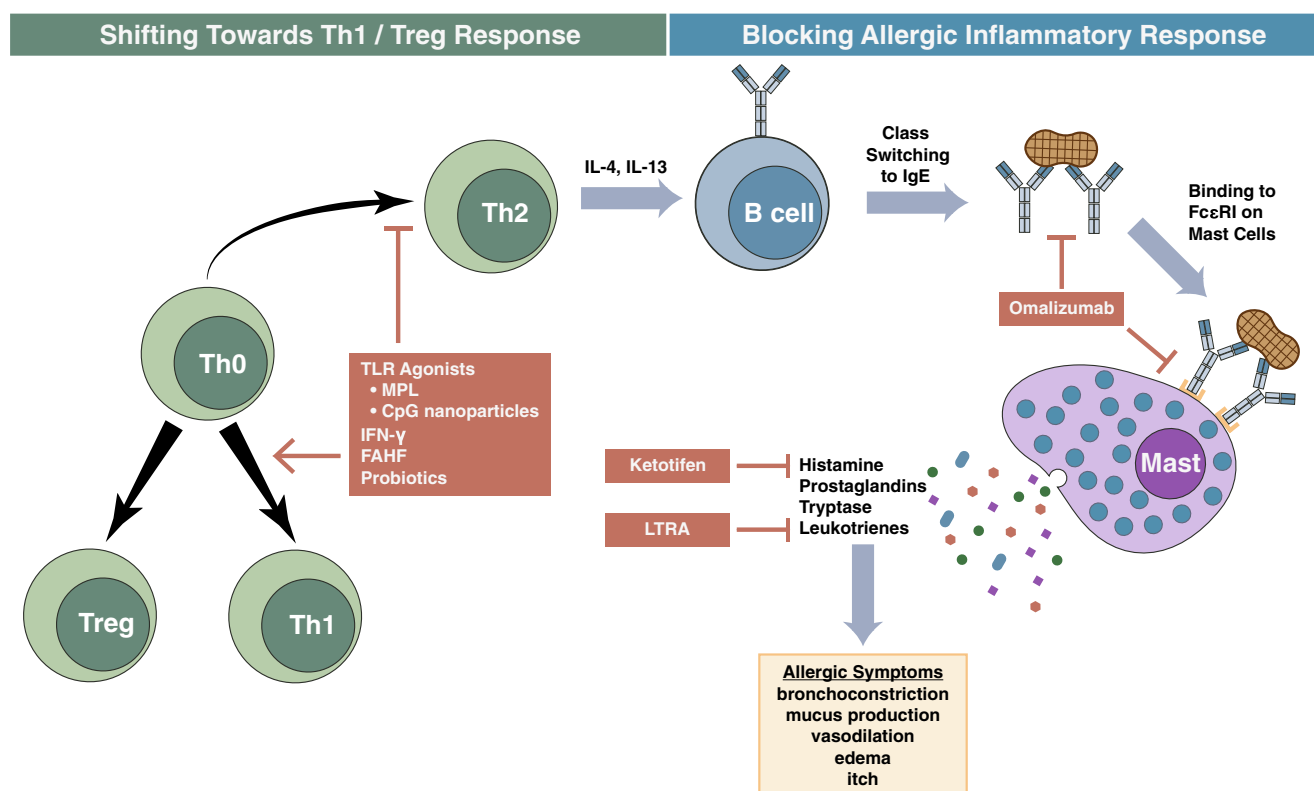


Fig. 1 Proposed mechanisms of adjunctive therapies for oral immunotherapy. Most adjunct therapies can be divided into one of two mechanisms. The first involves shifting the immune response from a Th2 polarized response to a Th1 and/or Treg dominated response (known for TLR agonists and IFN-gamma, and hypothesized for FAHF and probiotics). The second mechanism targets downstream aspects of the

allergic inflammatory response. Anti-IgE therapies, like omalizumab, block the receptor binding site on IgE, decreasing both free IgE and IgE receptors on mast cells. Other therapies like ketotifen and leukotriene receptor antagonists inhibit the actions of mast cell mediators like histamine and leukotrienes, respectively

(one dropped out due to abdominal symptoms), and nine of these (82%) were able to achieve the goal maintenance dose. Upon double-blind, placebo-controlled food challenge at week 24 of the study, all nine of the subjects who reached maintenance consumed 8000 mg of milk protein without symptoms. The rate of reactions was 1.6% during buildup and maintenance (reactions/total doses among all subjects), with all subjects reporting at least one symptom. The majority

of these reactions were mild, though there were three instances of epinephrine use among three of the subjects (27% of all subjects, and higher than proportions reported with OIT, though this comparison may be limited by differences in sites' usage of epinephrine). Sustained unresponsiveness was not evaluated. This pilot was the first to demonstrate the feasibility of a shorter buildup for OIT with a reaction rate comparable to other OIT studies.

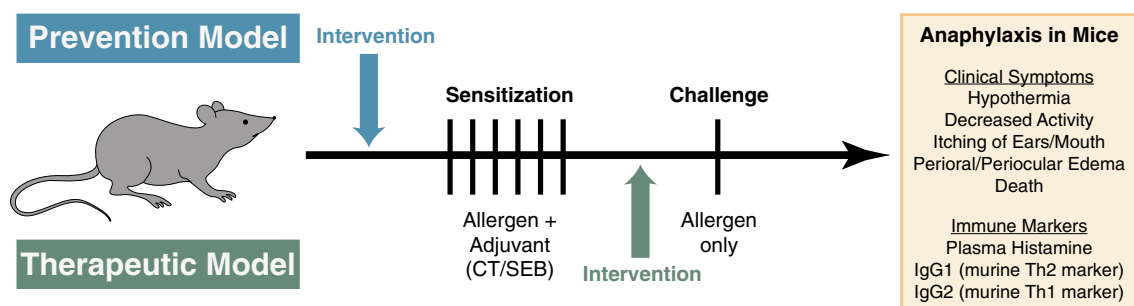


Fig. 2 Preventative and therapeutic mouse models of food allergy. The majority of preclinical studies assess the efficacy of a potential treatment using either a preventative or therapeutic murine model. These models generally use peanut or ovalbumin as an allergen and expose the mouse to the allergen with an adjuvant, such as cholera toxin (CT) or staphylococcal endotoxin B (SEB), during a process known as sensitization. The

allergic response is evaluated by administering a dose of allergen alone and monitoring for clinical signs of anaphylaxis or immune markers of a Th2 or Th1 response. In preventative models, the intervention is given prior to the sensitization process, whereas in therapeutic models, the intervention is given after sensitization

Following this pilot study, three studies reported on the use of omalizumab with OIT for other food allergens, peanut, egg, and milk [28–30]. The pilot study by Schneider et al. for peanut allergy evaluated omalizumab administration prior to and during OIT desensitization in 13 children with peanut allergy confirmed by entry challenge [28]. Participants received 12 weeks of omalizumab (dosed every 2–4 weeks), followed by a build-up phase over approximately 8 weeks with concurrent omalizumab dosing. Twelve of 13 participants reached the goal peanut protein dose of 2000 mg. Twelve weeks after stopping omalizumab, all 12 were challenged and consumed 4000 mg peanut protein without symptoms (11 passed a double-blind, placebo-controlled challenge, and one failed this challenge but passed an open challenge later). The rate of reactions during OIT was 2% (reactions/doses among all subjects). The majority of these reactions were mild, with five instances of epinephrine use among three subjects (23% of all subjects, similar to the proportion seen in the Nadeau et al. study). Sustained unresponsiveness was not evaluated. This study demonstrated that rapid desensitization could successfully be performed in other allergens as well, namely, peanut.

One case series of three egg allergic children reports their experience with omalizumab and OIT, specifically focusing on children who experienced increased allergic reactions after stopping omalizumab [29]. Lafuente et al. reported from their routine clinical practice and described two children who had previously failed egg OIT due to repeated vomiting after their daily doses during buildup, and a third where they had high clinical suspicion that she would not tolerate egg OIT due to with elevated specific IgE to egg. All three children received 2–3 months of omalizumab pretreatment, followed by desensitization with concurrent omalizumab for an additional 2–4 months. After 3–4 months off omalizumab, all children experienced a recurrence of allergic symptoms while maintaining an OIT dose they had previously been tolerating. Symptoms were primarily abdominal pain and vomiting, with cough reported for one child. These children likely represent a more sensitive subset of the egg-allergic population given high levels of sensitization and previous failure to tolerate OIT. It should be noted, however, that the recurrence of symptoms occurred after the typical 8–12-week follow-up time documented in the previous pilot studies, supporting the necessity of longer follow-up after discontinuing omalizumab.

In 2017, Burk et al. described a case series of their experience with eosinophilic esophagitis in 2 of 13 children enrolled in a phase I/II trial studying peanut OIT with omalizumab [31]. In this study, participants received 4 months of omalizumab pretreatment, which continued through OIT buildup (approximately 0.5–1 year), until 1 month of maintenance (goal 4000 mg peanut). Of the 13 enrolled, 6 (46%) passed a 10-g desensitization challenge and 4 (31%) passed the challenge after 2–4 weeks of peanut avoidance [32]. Both cases reported worsening gastrointestinal symptoms 4–

8 weeks after stopping omalizumab, and upon endoscopy, were diagnosed with eosinophilic esophagitis [31]. Both resolved clinically and histologically after stopping OIT, but over 1 year after stopping OIT, the first subject had a recurrence despite having had no peanut ingestions. In retrospect, the subject had noted intermittent dysphagia a year prior to starting OIT. The rate of eosinophilic esophagitis in this trial (15%) is higher than the 2–3% reported in the literature in association with OIT [9, 33]. However, the double-blind, placebo-controlled trials of OIT with omalizumab discussed below showed lower rates of eosinophilic esophagitis, similar to those reported in the literature [34, 35]. As Burk et al. note, because symptoms often resolve after stopping OIT, many patients do not undergo endoscopy so the true rates of eosinophilic esophagitis in these populations may be underestimated [31]. They hypothesize that omalizumab may mask early gastrointestinal symptoms that generally lead to withdrawal from OIT. Larger studies, adequately powered to address side effects, will need to explore any potential association between omalizumab and severe treatment-limiting gastrointestinal side effects or eosinophilic esophagitis.

A report by Martorell-Calatayud et al. in 2016 described their experience with omalizumab in 14 egg or milk allergic participants who had failed conventional OIT due to multi-system allergic reactions or anaphylaxis [30]. Participants were given omalizumab for 8 weeks, followed by desensitization to a goal of 1800 mg of egg protein or 6600 mg of milk protein over approximately 18 weeks. Open food challenge was performed, and subjects transitioned to maintenance therapy with 2 additional months of omalizumab. Due to concerns about allergen thresholds worsening while on maintenance OIT due to the discontinuation of omalizumab, approximately 1 month after stopping omalizumab, they repeated desensitization starting from 25% of the maintenance dose which all subjects tolerated. They followed participants for a total of 12 months after the last omalizumab dose. Of the 14 patients, 6 (60% of the milk, and 33% of the egg allergic patients) developed anaphylactic symptoms 2.5–4 months after stopping omalizumab. Though their definition of relapse based on anaphylactic reactions differed from the previous paper which focused on recurrent gastrointestinal symptoms, both demonstrated increased adverse reactions approximately 3–4 months after stopping omalizumab among patients who had failed conventional OIT, supporting the need for prolonged follow-up post-omalizumab.

Double-Blind, Placebo-Controlled Trials of Omalizumab With OIT

Two double-blind randomized placebo-controlled multicenter trials have been performed studying omalizumab treatment with OIT in milk and peanut [34, 35]. In the milk trial performed by Wood et al., 57 participants with milk allergy

confirmed by challenge were randomized to either omalizumab ($n = 27$) or placebo ($n = 28$) injections for 4 months, then received OIT desensitization for 22–40 weeks, followed by 1–1.5 years of maintenance at a goal dose of 3.8 g of milk protein [34]. Omalizumab was then discontinued followed by an oral food challenge to assess desensitization. In each arm, 26 patients tolerated desensitization and maintenance, and 89% of the omalizumab-treated subjects and 71% of the placebo-treated subjects tolerated 10,000 mg of milk protein without symptoms. Patients later stopped OIT for 2 months and were reassessed by challenge, at which point 48% of omalizumab-treated subjects and 36% of placebo-treated patients showed sustained unresponsiveness to milk allergen. There were no statistically significant differences in efficacy outcomes between the groups. The omalizumab group did achieve maintenance faster than the placebo group (25.9 versus 30 weeks, $p = 0.01$). Furthermore, the omalizumab group experienced lower rates of adverse reactions, 2.1% (reactions/doses of OIT) in the omalizumab group versus 16.1% in the placebo group ($p = 0.0005$). Similarly, the omalizumab group experienced lower rates of dose-related reactions requiring treatment ($p = 0.008$), and a trend of fewer reactions requiring epinephrine (omalizumab, 2 doses among 2 subjects, versus placebo, 18 doses among 9 subjects; $p = 0.052$). This was the first study to evaluate sustained unresponsiveness in the setting of OIT combined with omalizumab. Based on these findings, it was determined that omalizumab improved the safety profile of OIT but had no effect on the efficacy of OIT (48.1% in omalizumab group versus 35.7% in placebo group).

The trial conducted by MacGinnitie et al. randomized 37 participants with peanut allergy confirmed by a baseline peanut challenge to omalizumab ($n = 29$) or placebo ($n = 8$) for 12 weeks [35]. Twenty-three subjects (79%) in the omalizumab-treated group achieved the maximum target dose for the day 1 desensitization (250 mg) versus only 1 subject (13%) of the placebo group ($p < 0.01$). Participants then underwent a buildup phase of 8 weeks, increasing to a goal maintenance dose of 2000 mg peanut protein. Omalizumab was then discontinued, and participants continued to receive maintenance peanut OIT dosing for 12 weeks, after which they underwent a food challenge to 4000 mg of peanut protein. During this challenge, to assess desensitization, 22 subjects (76%) from the omalizumab group passed, and 13% from the placebo group passed. Regarding safety, the omalizumab group experienced reactions after 7.8% of doses compared to 16.8% of doses in the placebo arm, though not a statistically significant difference. Epinephrine was administered four times in the omalizumab arm and three times in the placebo arm. Sustained unresponsiveness was not evaluated in this study. Two participants in the omalizumab arm and one participant from the placebo arm withdrew due to symptoms consistent with eosinophilic esophagitis; two of these three

had confirmation of eosinophilic esophagitis with biopsy. Overall, this trial demonstrates that omalizumab can facilitate a faster OIT buildup, with a rapid initial day dose escalation, with similar safety profiles compared to placebo injections.

Omalizumab with OIT for Multiple Foods

Omalizumab has also been used as an adjunct to multiple allergen OIT, with the first phase I study published in 2014 by Bégin et al. [36]. This trial enrolled 25 subjects, all with multiple food allergies, including milk, egg, peanut, tree nuts, wheat, soy, and sesame, confirmed by double-blind, placebo-controlled food challenge. Omalizumab was administered for 8 weeks, followed by desensitization to up to five allergens over a median time of 18 weeks, with a goal maintenance dose of 4000 mg of protein per allergen. After 8 weeks of dose escalation, omalizumab was discontinued. Of the 25 subjects enrolled, 88% remained in the study, and the remaining 3 withdrew due to medication non-adherence. No food challenges to test desensitization or sustained unresponsiveness were performed. The reaction rate was 5.3% of all OIT doses administered. This study served as a proof of concept that omalizumab could facilitate the administration of multiple allergen OIT at doses equivalent to those of single food OIT.

Mechanistic Studies of Omalizumab with OIT

A number of studies have examined the immunologic changes associated with omalizumab as an adjunct to OIT [34, 37–39], with the studies based on randomized controlled trials being notable for their ability to directly compare effects of omalizumab on the desensitization induced by OIT. Wood et al., in their randomized controlled trial of omalizumab with milk OIT described above, compared serologic changes in IgE, IgG, and IgG4 to milk, casein, and beta-lactoglobulin in the omalizumab versus placebo groups [34]. They found no significant differences between the groups in milk component IgG or IgG4 levels, and while the omalizumab group had higher milk and casein-specific IgE at month 4, levels were similarly reduced in both groups by the end of therapy.

The mechanistic study by Frischmeyer-Guerrero et al. examined changes in basophil reactivity and biomarkers associated with safety and efficacy outcomes between groups receiving OIT with and without omalizumab. Using samples from the Woods et al. study, they measured basophil responsiveness based on %CD63 expression, and found that the omalizumab group not only had lower basophil CD63 expression immediately after omalizumab administration (month 4) but also throughout the administration of OIT, as compared to the placebo arm. In both groups, basophil responsiveness returned to baseline upon stopping OIT, showing that omalizumab effects on basophil reactivity did not persist beyond the duration of therapy. Examination of milk-specific

Tregs showed no increases over the course of therapy in either group. Comparing baseline basophil responsiveness with rates of OIT-related allergic adverse events identified a subgroup of individuals with CD63 expression greater than 40% who were most likely to benefit from the administration of omalizumab. In contrast, subjects with CD63 expression lower than 40% had similar rates of allergic adverse events regardless of whether they received omalizumab. Furthermore, they defined a subgroup of patients based on an elevated ratio of %CD63 expression on basophils stimulated by milk over basophils stimulated by anti-IgE; within this subgroup, proportions achieving sustained unresponsiveness were significantly higher in the omalizumab arm than the placebo arm. The authors hypothesize that targeting omalizumab use to this subgroup of patients may show differences in sustained unresponsiveness outcomes that were not observed in the primary outcome in the original study. While these findings will require validation in larger cohorts, they suggest that it may be possible to target the more expensive biologic therapy towards individuals most likely to benefit from its use, with respect to both improved safety and sustained unresponsiveness.

In summary, a number of clinical trials have examined the use of omalizumab with OIT. Omalizumab as an adjunctive therapy clearly allows for faster dose escalations and lower adverse event rates. The concern raised by Burk et al. as discussed above, namely, that omalizumab masks early gastrointestinal symptoms that would ordinarily lead to dropout and therefore results in more severe gastrointestinal pathology, may merit further study. Targeted use of omalizumab for multi-allergen immunotherapy or rescuing individuals who failed traditional OIT may be ideal, but this will require further study in larger prospectively designed randomized controlled trials. Basophil reactivity may prove useful in targeting use of omalizumab towards populations most likely to benefit, which would be particularly important given the cost of biologic therapy, but this also will require further study. Initial studies indicate that omalizumab has no effect on the development of sustained unresponsiveness, but additional studies targeting omalizumab to certain subgroups of food allergy patients will be necessary to fully address this question.

Toll-Like Receptor Adjuvants

Toll-Like Receptors

Toll-like receptors (TLRs) encompass a variety of receptors that bind conserved motifs found primarily in bacterial and viral pathogens [40]. TLR signaling pathways not only shift the adaptive immune system towards a Th1 response but also induce Treg cells that may suppress the Th2 imbalance characteristic of allergic diseases [40, 41]. Much of the research investigating TLRs as potential adjuvants for allergen

immunotherapy has focused on TLR-9, an intracellular receptor that recognizes CpG oligonucleotide motifs, and TLR4, an extracellular receptor that binds lipopolysaccharides from Gram-negative bacteria [40, 42].

TLR-9 Agonists: CpG Oligonucleotides

CpG oligonucleotides, short DNA motifs containing the unmethylated cytosine-phosphate-guanine (CpG) dinucleotide, are abundant in bacteria and viruses and are known to stimulate the immune system by binding toll-like receptor 9 (TLR9) [43]. Early research in murine models of food allergy demonstrated that administration of CpG could both prevent sensitization to peanut allergen and decrease reactivity to peanut allergen in treatment models of mice with established food allergy [44–46]. In 2001, Srivastava et al. explored the use of these CpG motifs using a construct linking immunostimulatory sequences (ISS) containing CpGs with one of the major peanut protein components Ara h 2 [47, 48]. Mice were pretreated with either the ISS-linked Ara h 2 or ISS-linked to a ragweed protein, and then, they were sensitized to peanut. Upon challenge to peanut, the mice immunized with ISS-linked Ara h 2 had milder allergic reactions and lower plasma histamine levels than those immunized with the ISS-linked ragweed construct. No difference was noted, however, in the peanut-specific IgE or IgG1 levels between the groups.

In 2013, Kulis et al. reported on work combining CpG administration with allergen immunotherapy in both prevention and treatment murine models of food allergy [49]. Immunotherapy was administered to peanut allergic mice over 4 weeks with three injections of peanut a week, followed by challenge to peanut. They evaluated three types of CpG motifs coadministered with peanut immunotherapy, compared with peanut immunotherapy alone and placebo. Type B CpG motifs, known for their effects on B cell proliferation and antibody secretion, were able to both prevent sensitization and decrease anaphylactic reactions when coadministered with immunotherapy, in comparison with the other two treatment groups. The mice receiving type B CpG and immunotherapy also had significantly higher interferon-gamma recall response and peanut IgG2a antibodies after treatment, compared to immunotherapy alone and placebo. These studies demonstrate the utility of CpG immunostimulation in reducing allergic reactions to peanut in murine models. Given the mitigating effects of CpG administration in both the sensitization and treatment of peanut allergy, newer preclinical research has focused on novel delivery methods for this treatment, specifically through nanoparticles, as discussed below. While there are no CpG based human trials in food allergy currently, aeroallergen immunotherapy trials using CpG conjugated with ragweed have been performed with some improvement in nasal inflammatory response though no difference in

patient-reported symptoms or medication use [50]. Also, CpG oligodinucleotides are being studied in phase I/II trials in concert with vaccines and a variety of cancers.

TLR-4 Agonists: MPL and GLA

Monophosphoryl lipid A (MPL) is a form of LPS derived from attenuated *Salmonella* that activates the TLR-4 signaling pathway [51]. In Europe and the USA, phase I/II/III trials are currently investigating MPL as an adjuvant to sublingual and subcutaneous aeroallergen immunotherapy for a variety of allergens including grass, ragweed, tree pollen, and dust mite [52]. A number of studies of MPL, including double-blind, randomized placebo-controlled trials, have demonstrated efficacy and safety in allergen immunotherapy for allergic rhinitis and asthma in adults and children as young as 6 years old [40, 53–55]. One study by Patel et al. of 228 patients with ragweed allergy compared ragweed subcutaneous immunotherapy, with or without MPL, to placebo, using an ultra-short course involving four injections over 3 weeks [54]. The ragweed and MPL group experienced higher response rates than both the placebo arm and the ragweed immunotherapy only arm, with improved quality of life and increased IgG1 and IgG4 production upon ragweed exposure. The ragweed immunotherapy only arm, however, was given the same ultra-short course administered to the ragweed and MPL group, which is not the traditional length of therapy required for optimum efficacy of ragweed subcutaneous therapy. Randomized controlled trials comparing the ultra-short course of MPL and ragweed immunotherapy with the traditional long course of ragweed immunotherapy alone would be necessary to determine if there were true differences in long-term efficacy. However, this study provides proof of concept that MPLs can be employed to shorten the onerous buildup period of immunotherapy and may have some utility in food allergen immunotherapy where the majority of allergic adverse events are experienced during the dose escalation phase.

Based on the success of MPL, a synthetic version, glucopyranosyl lipid A (GLA), is being investigated for a number of allergic diseases, including food allergy [56–58]. Preliminary work by Soos et al. examined therapeutic murine models of peanut allergy, where peanut-sensitized mice were given either sublingual peanut immunotherapy, with or without GLA, or placebo treatment for 1 month and then challenged to peanut [57]. The group receiving peanut immunotherapy with GLA had significantly lower anaphylaxis scores than placebo, whereas the peanut immunotherapy without GLA group had no difference from placebo. To assess the role of GLA in human cells, they extracted peripheral blood mononuclear cells from peanut allergic patients, incubated them with peanut extract and varying doses of GLA, and measured CD4⁺ T cell proliferation. Here, they found dose-dependent inhibition of peanut-specific helper T cell proliferation by

GLA. These data support a role for GLA as a potential adjuvant for food allergen immunotherapy, and phase I trials studying GLA co-administration with peanut OIT are ongoing.

Novel Delivery Methods—Nanoparticles

Nanoparticles

Nanoparticles represent a type of particulate delivery systems which are of interest in the study of food allergen immunotherapeutics due to the high rate of allergic side effects experienced by participants on OIT [59]. Nanoparticles not only shield the allergens from degradation by enzymatic breakdown in the gastrointestinal tract but may also increase the efficiency of antigen uptake [60]. For drug delivery, biodegradable polymers are the most common carrier type, and can include natural polymers like chitin, derived from crustaceans, or synthetic polymers like polyesters or polyanhydrides [59]. Antigens are either loaded inside the nanoparticle or adsorbed into the outer layer of the nanoparticle. All of the research on nanoparticles for food allergy remains in the pre-clinical stages, evaluating both prevention and treatment murine models.

Nanoparticles and Food Allergy

Some of the earliest work with nanoparticles and food allergy was reported in 1999 by Roy et al., using a murine prevention model to assess orally administered chitosan nanoparticles as a delivery vehicle for plasmid DNA with a gene for Ara h 2 [61]. Mice were immunized with either oral chitosan-Ara h 2 nanoparticles, Ara h 2 alone, or no immunization. All mice were then sensitized with peanut by intraperitoneal and oral routes, and then challenged with recombinant Ara h 2 to evaluate their allergic reactivity. After immunization, the nanoparticle immunized mice had increased secretory fecal IgA and anti-Arah2 IgG2a, suggesting a Th1-type response, that was absent in the other mice. Upon challenge, the nanoparticle immunized mice had less severe and delayed anaphylactic reactions compared to non-immunized mice and mice immunized with Ara h 2 DNA alone. Of note, these experiments were repeated with a group of mice receiving two doses of nanoparticles, and no dose response was noted.

In 2007, Gomez et al. published their work using orally administered polyanhydride nanoparticles loaded with ovalbumin (OVA) in murine treatment models of food allergy [62]. Mice were initially sensitized to OVA, and then immunized by oral gavage with one of the four treatments: nanoparticles containing OVA, nanoparticles containing OVA and LPS, nanoparticles containing OVA and coated with LPS, and free OVA without nanoparticles. OVA-specific IgG1 and IgG2a

levels were highest in the group receiving nanoparticles containing only OVA, though increased in all groups that received nanoparticles containing OVA. After 1 month, animals were challenged with OVA intraperitoneally, with the no mortality in the nanoparticle containing OVA only group, 20% mortality in the nanoparticle containing both OVA and LPS group, mortality of 60% in the control group, and the highest mortality of 80% in the group receiving nanoparticles coated with LPS, containing OVA. While the authors initially hypothesized that LPS would act as an adjuvant improving efficacy, further experiments revealed that the addition of LPS induced faster release of the loaded OVA protein, potentially explaining the poorer performance of the LPS-related nanoparticles. Symptoms were milder in the groups receiving nanoparticles with OVA but these differences were not significant. Overall, this study was the first to evaluate nanoparticles in a treatment model of food allergy and showed orally administered OVA containing nanoparticle immunotherapy was successful in decreasing mortality in a murine OVA-sensitized allergy model.

Reboucas et al. in 2014 aimed to compare a variety of preparations of polyanhydride nanoparticles to determine any effects on the sensitization of wild-type mice in a food allergy prevention model [63]. They compared nine total groups: six nanoparticle groups, defined by their method of drying (lyophilized versus spray-dried) and the nanoparticle contents (unloaded, loaded with raw peanut, or loaded with roasted peanut), and three controls (no intervention, raw peanut protein without nanoparticles, roasted peanut protein without nanoparticles). The peanut nanoparticles were associated with higher peanut-specific IgA than peanut alone. The study found no difference between the use of raw versus roasted peanut protein, but did note that the spray-dried nanoparticles induced higher IgG2a and lower IgE levels, suggesting a Th1 response, while the lyophilized nanoparticles induced higher levels of IgG1 suggesting a Th2 response. This enhancement of a Th1 response with the spray-dried nanoparticles was thought to be due to the higher nanoparticle stability from the spray-drying process, resulting in increased adjuvant effects and poorer allergen release. As the authors note, the study was designed to optimize variables associated with nanoparticle delivery, but these variables required further evaluation in treatment model of allergic mice.

Nanoparticles and CpG

Given the promising immunomodulatory effects of CpG, two more recent studies evaluated the use of CpG-coated nanoparticles loaded with peanut. Pali-Scholl et al. in 2013 used protamine nanoparticles loaded with peanut to evaluate differences in sensitization of wild-type mice as a prevention model [64]. They compared five treatments administered subcutaneously for 1 month: Ara h 2 alone, Ara h 2 with alum as an

adjuvant, nanoparticles loaded with Ara h 2, nanoparticles alone, and control mice immunized with buffer solution. Compared with the mice immunized with Ara h 2 alone, the nanoparticles loaded with Ara h 2 had increased IgG2a antibodies, decreased Ara h 2-specific IgE, and a low ratio of IL-5/interferon-gamma, all suggestive of a decreased Th2 immune response. The mice receiving nanoparticles with Ara h 2 did develop some reactivity as documented by Ara h 2-specific skin test. In this sensitization mouse model, CpG-coated nanoparticles loaded with peanut protein showed evidence of suppression of the Th2 response, presumably due to the Th1-stimulating effects of the CpG-coated nanoparticles.

In 2016, Srivastava et al. reported their findings administering CpG-coated poly(lactic co-glycolic acid) nanoparticles loaded with peanut protein in a treatment model of peanut allergy [65]. Mice were sensitized intragastrically with peanut and cholera toxin. Mice were then randomized one of six treatment groups involving weekly gavage for 4 weeks: CpG nanoparticles containing peanut, non-coated nanoparticles containing peanut, CpG-nanoparticles alone, non-coated nanoparticles alone, peanut alone, or buffer solution alone. Mice then had five oral peanut challenges monthly, and consistently, the mice with the CpG nanoparticles loaded with peanut had the highest level of protection, with the lowest anaphylaxis symptom scores, lowest plasma histamine levels, and were protected from the hypothermia usually associated with anaphylaxis. Of note, the peanut allergic mice in all of the other treatment groups, including those receiving non-coated nanoparticles with peanut as well as CpG-coated nanoparticles, had similar severity of anaphylaxis, plasma histamine levels, and hypothermia when challenged to peanut, which was likely due to the fact that the doses of peanut administered during nanoparticle treatment were very low (micrograms) compared to the milligram doses normally administered in successful murine OIT. The group receiving CpG nanoparticles with peanut was the only treatment arm to show significantly lower levels of peanut-specific IgE, lower peanut-specific IgG1, and higher IgG2a, all consistent with a Th1-driven response. After the challenges, the mice were killed, splenocytes were harvested, stimulated with peanut, and cytokine levels were measured. The CpG nanoparticles with peanut group had the lowest levels of Th2 cytokines IL-4, IL-5, and IL-13, and the highest levels of interferon-gamma (no significant differences in IL-10 or TGF-beta). While the CpG nanoparticles containing peanut showed promising shifts towards Th1 responses and protection from allergic reactivity, the authors noted concerns that the CpG sequences active in stimulating a murine immune system may not act similarly in humans. Accordingly, they prepared nanoparticles that were assembled with total *Escherichia coli* membranes and loaded with peanut protein and sheared *E. coli* DNA, with the goal of binding a larger variety of toll-like receptors. These nanoparticles performed similarly to the CpG nanoparticles containing

peanut. This was the first study to show efficacy of nanoparticles in a murine treatment model of peanut allergy, with a clear prolonged protection lasting months after stopping immunotherapy in both the CpG-coated nanoparticles containing peanut and the *E. coli* nanoparticles containing peanut. Future studies will need to include a comparison of nanoparticle therapy delivering microgram doses of allergen to standard murine OIT with milligrams of allergen to compare safety and efficacy. To date, CpG-coated nanoparticles have not been studied in humans, but nanoparticles loaded with CpG have been studied in melanoma [66].

Nanoparticles and Rapamycin

Recent studies have investigated nanoparticles containing rapamycin, also known as sirolimus, an inhibitor of the mammalian target of rapamycin (mTOR) pathway that is known to induce antigen-specific immune tolerance [67]. Yamaki and Yoshino demonstrated the efficacy of rapamycin administration in attenuating allergic responses in both preventative and therapeutic murine models of food allergy [68]. Maldonado et al. evaluated the efficacy of poly(lactide-coglycolide) nanoparticles containing egg white protein OVA and rapamycin, in a sensitization murine model. Mice were pretreated with either the nanoparticles with OVA and rapamycin or placebo injections and sensitized intraperitoneally to OVA over 2 weeks. Upon challenge to OVA, the mice receiving nanoparticle treatment with rapamycin and OVA had lower anaphylaxis scores, reduced hind limb swelling, and decreased anti-OVA IgE, compared to the mice who received no nanoparticles. This experiment lacked controls showing the effect of nanoparticles with OVA alone or empty nanoparticles for comparison. Based on this preclinical work, nanoparticles containing rapamycin and allergen reduced reactivity when administered prior to sensitization. However, further evaluation is necessary to determine if rapamycin has utility as an adjunct to allergen containing nanoparticles. Phase I and II trials are underway evaluating nanoparticles containing rapamycin in a variety of cancers, though there are no clinical trials for allergy at this time.

Probiotics

Probiotics and Allergy

Research in probiotics and allergy has garnered much interest in the last two decades, and while the use of probiotics to prevent allergy remains controversial based on conflicting research, in vitro work does show the strong influence of the microbiome on the development of immune tolerance [69, 70]. Work in mouse models has shown that raising mice in sterile environments results in decreased IL-10-producing T

regulatory cells and an inability to develop oral tolerance [71, 72]. From a therapeutic perspective, treatment with certain bacterial species can promote the development of IL-17-producing T cells and T regulatory cells in mice [73, 74]. Enrichment of specific bacteria, particularly *Clostridia*, has been shown in murine models to protect against development of food allergen sensitization and even induce Treg responses [75]. Combined with the early evidence of bacterial adjuvants enhancing the efficacy of immunotherapy, this work supports the hypothesis that probiotics could enhance the tolerogenic effects of OIT [51, 76].

Probiotics and OIT

Tang et al. published their findings in 2015 from a double-blind, placebo-controlled randomized trial of probiotics with peanut OIT [77]. They randomized 62 children peanut allergic children, confirmed by history and peanut-specific testing, to either peanut OIT with the probiotic *Lactobacillus rhamnosus* GG or the placebo arm with placebo probiotic and placebo OIT. *L. rhamnosus* GG was selected because of prior reports supporting its ability to induce Th1 and T regulatory responses in allergic disease [78–80]. Their OIT protocol included 1-day modified rush induction, 8 months of buildup dose escalation, and 10 months of maintenance (goal dose 2000 mg peanut protein). All treatment was stopped after 18 months, and sustained unresponsiveness was measured by food challenge to 4 g peanut protein occurring 2–5 weeks after stopping therapy. In the active arm, 82% of subjects achieved the primary outcome of sustained unresponsiveness, compared to 4% of the placebo subjects. The study is limited by the absence of an OIT-only arm to compare the rate of sustained unresponsiveness. While the active arm showed a higher rate of sustained unresponsiveness than has been generally reported in the literature, this may be due to multiple variables, including the early assessment of sustained unresponsiveness at 2 weeks, compared with prior work showing approximately 50% with sustained unresponsiveness assessed at 4 weeks after peanut OIT [81].

In 2017, the same group published a follow-up report of outcomes in this cohort of peanut allergic individuals [82]. Upon completion of the first study, those who passed a sustained unresponsiveness challenge were advised to consume peanut ad libitum with no guidance regarding frequency or dose. Those who failed this challenge were advised to avoid peanut. The authors note that probiotics were likely not continued at the doses administered during the trial, as the doses were much higher than those contained in normal servings of commercial products. The follow-up study enrolled 24 participants from each arm, and from the active arm, 16 subjects (67%) were still eating peanut compared to 4% from the placebo arm. Twelve of these subjects (52%) ate > 2 g of peanut protein at a time and 11 (46%) consumed peanut at least once

a week. In the active arm, four participants (17%) experienced at least one allergic reaction to peanut during the follow-up period, compared to six (25%) in the placebo group. Most of these reactions occurred 1–5 years after the end of the trial, though none were classified as anaphylaxis and epinephrine was not administered. Participants were then asked to avoid peanuts for 8 weeks and challenged to assess sustained unresponsiveness. From the active arm, 12 subjects agreed to participate, and of these, 7 (58%) passed an 8-week sustained unresponsiveness challenge, compared to 1 (7%) of 15 subjects challenged from the placebo arm. It is notable that over half of the subjects were able to demonstrate sustained unresponsiveness despite the lack of a strict ingestion schedule. While the analysis is limited by the lack of an entry challenge to confirm peanut allergy in the original cohort, the 60% who passed a sustained unresponsiveness challenge after an irregular ingestion schedule are much higher than the 20% rate of naturally outgrowing peanut allergy reported in the literature and are consistent with reported rates of sustained unresponsiveness after OIT [83]. However, it remains unclear if this is due to the effects of probiotic administration or the possibility that a strict ingestion schedule is not necessary to maintain the protection from OIT. Future studies to assess the utility of probiotics in conjunction with OIT will need to compare the administration of OIT with and without probiotics in a large randomized controlled trial, ideally with a similar 4–8-week period of avoidance to evaluate the presence of sustained unresponsiveness. Further study of the immunomodulatory effects of specific strains of probiotics on OIT will also be necessary. The authors have indicated that a follow-up study of probiotics with OIT, including a direct comparison of OIT without probiotics, is in progress [84].

Traditional Chinese Medicine for Food Allergy

Another novel approach to food allergy treatment is the use of herbal therapy based on traditional Chinese medicine. A nine-herb formula, named the food allergy herbal formula 2 (FAHF-2), has been developed and tested in murine models of food allergy and is now in clinical trials [85]. The formula is based on a traditional Chinese medicine (Wu Mei Wan) that was originally used for the treatment of intestinal parasite infections [86] and has also been demonstrated to be effective for gastroenteritis and asthma in clinical studies in China [87]. This formula was modified for potential use in food allergy by adding Ling-Zhi, an herb that has anti-allergy and anti-inflammatory properties [88, 89], and removal of two herbs (Xi-Xin and Zhi-Fu-Zi) that are potentially toxic if processed improperly.

The safety and efficacy of FAHF-2 has been demonstrated in murine models of peanut-induced anaphylaxis and multiple food allergies. Early experiments used C3H/HeJ mice that

were sensitized with peanut and cholera toxin [85]. These mice were treated with FAHF-2 or sham twice a day for 7 weeks and then underwent a series of food challenges. The dose of FAHF-2 was determined based on the equivalent effective daily dose from humans based on body surface area. In post-treatment challenges, FAHF-2-treated mice were not reactive upon peanut exposure, with symptom scores, body temperatures, and plasma histamine levels that were no different from naive mice. In contrast, sham-treated mice had severe symptoms, significant drops in body temperature, and elevated plasma histamine levels, consistent with anaphylaxis to peanut. The effects of FAHF-2 are prolonged despite discontinuation of treatment, as the treated group continued to have a lack of or mild response to peanut exposure for nearly 6 months. The protective effects of FAHF-2 were seen both when treatment was administered early in the peanut sensitization process as well as when treatment was given after peanut allergy had been established [90, 91]. Although the treatment effect wanes over time, repeat treatment with FAHF-2 can re-establish the protection from peanut-induced anaphylaxis.

Immunological changes of decreased peanut-specific IgE and increased peanut-specific IgG2a levels were noted in parallel to the clinical effects seen at challenge. Moreover, significant reductions in production of Th2 cytokines (IL-4, IL-5, and IL-13) by cells of the mesenteric lymph node and splenocytes were noted to be sustained up to week 50. These changes were not due to general immunosuppression as enhanced production of interferon-gamma was also seen. Additional changes noted included reduction in peripheral blood basophils and peritoneal mast cells [92]. A decrease in mast cell FcεRI expression, but not basophil FcεRI expression, was observed. Using a murine mast cell line (MC/9 cells), FAHF-2 has inhibitory effects on IgE-mediated proliferation and degranulation (using anti-DNP IgE). These findings suggest a direct effect of FAHF-2 on basophil and mast cell suppression.

The effects of FAHF-2 are not specific to peanut as similar results were seen using a murine model of multiple food allergies (to peanut, egg, and fish) [93]. At the post-treatment challenges for each allergen, FAHF-2-treated mice were not reactive, with no symptoms, no change in body temperature, and no change in plasma histamine level. Decreased allergen-specific IgE levels and increased specific IgG2a levels were observed, along with significant reductions in production of Th2 cytokines and increased IFN-gamma production by splenocytes and mesenteric lymph node cells.

Two phase I clinical trials have been completed. The short-term phase I trial was a double-blind, placebo-controlled dose escalation study that enrolled subjects between 12 and 45 years of age who had documented peanut, tree nut, fish, and/or shellfish allergy (based on history and positive skin test and/or detectable food-specific IgE) [94]. All three doses tested (4,

6, or 12 tablets three times a day for 7 days) were well tolerated. Subjects experienced no significant adverse effects, based on clinical report, complete blood count and complete metabolic profile, urinalysis, electrocardiogram, and pulmonary function tests.

The long-term safety and tolerability of FAHF-2 was subsequently assessed in an extended phase I trial [95]. This was an open-label study where subjects received six tablets of FAHF-2 three times a day for 6 months. Fourteen of the 18 (77.8%) enrolled subjects completed the study, demonstrating that prolonged treatment with FAHF-2 was well tolerated with no significant adverse effects. One subject withdrew due to adverse effects of transient abdominal complaints without vomiting or diarrhea. The remaining withdrew due to difficulties with time commitment and adherence ($n = 2$) and for pregnancy ($n = 1$). Although no efficacy parameters were assessed in this study, exploratory studies showed suppression of allergen-stimulated basophil activation (CD63 expression) and a trend towards reduction in peripheral blood basophil and eosinophil percentages after 6 months of FAHF-2 treatment.

A phase II clinical trial was performed to assess the efficacy of monotherapy with FAHF-2 for the treatment of food allergies [96]. In this multi-center, randomized, double-blind, placebo-controlled study, 68 subjects, ages 12–45 years, with peanut, tree nut, sesame, fish, or shellfish allergy were enrolled. After baseline double-blind, placebo-controlled food challenge (DBPCFC) to verify allergy, subjects were randomized (2:1) to receive 10 tablets of FAHF-2 or placebo three times a day for 6 months while continuing allergen avoidance. DBPCFC was performed for all subjects at the end of the treatment period. The results indicated that while FAHF-2 was well tolerated with no serious adverse events, efficacy was not demonstrated at this dose and duration of treatment. A notable limitation of the study was the high daily dose, which contributed to dropouts (13%) as well as lower than desired adherence rates (44% of subjects had low adherence for at least one third of the study).

In order to address the burden of a high daily dose, a refined formulation was developed using an additional butanol purification step (B-FAHF-2) [97]. The refined formula produced comparable effects as FAHF-2 and had an equivalent safety profile and persistence of effect in the murine model. Furthermore, murine experiments using B-FAHF-2 in combination with multiple allergen OIT demonstrated treatment with B-FAHF-2 with OIT resulted in fewer adverse reactions and longer post-treatment protection than OIT alone [98]. Currently, a phase II randomized, double-blinded, placebo-controlled study investigating the safety and efficacy of this formula in combination with multi-allergen OIT and omalizumab in the treatment of multiple food allergies is ongoing ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02879006) identifier NCT02879006).

Other Adjunctive Therapies

Interferon-Gamma

Other adjunctive therapies to OIT include interferon-gamma, ketotifen, and leukotriene receptor antagonists. Dysregulation of the cytokine interferon gamma has been implicated in food allergy by a study comparing children with cow's milk allergy to healthy controls [99]. The children with cow's milk allergy confirmed by oral challenge had undetectable levels of interferon gamma compared to healthy children; however, those who outgrew the allergy developed enhanced production of interferon-gamma. Based on this work, the first study evaluating interferon-gamma was performed by Noh and Lee in 2009 and enrolled 25 subjects with challenge-confirmed IgE mediated allergies to milk, eggs, or wheat [100]. The subjects were split into four arms: interferon-gamma and OIT ($n = 10$), OIT alone ($n = 5$), interferon-gamma treatment alone ($n = 5$), and no therapy ($n = 5$). Of note, three children from the interferon-gamma and OIT arm had multiple food allergies, which were classified as being treated as interferon-only treated for those allergens. In this study, OIT differed from other trials; in that, there was no modified rush treatment, and the dose was administered 6 days a week, with buildup occurring only if the previous day's dose had been administered without adverse reactions, allowing rapid dose escalations as frequent as every day. Target doses were 100 ml of milk (3.3 g milk protein), 25 g of eggs (3.2 g egg protein), and 50 g of wheat noodles (protein content unknown). Interferon-gamma injections were administered subcutaneously every weekday prior to the doses of OIT. Only the children who received interferon-gamma and OIT were able to tolerate buildup to the target maintenance dose of food allergen; those subjects in the OIT only arm all stopped OIT due to frequent and severe anaphylactic reactions. After a final food challenge equivalent to a normal serving size of the allergen to assess desensitization, only the OIT + interferon-gamma group showed improvement in severity of allergic reaction. The remaining three groups had similar symptoms to their original challenge at the end of therapy, including the OIT-only group. In summary, the study administered a relatively rapid dose escalation without a 1-day modified rush desensitization, which was tolerated by those receiving daily interferon-gamma injections, but not by those receiving OIT alone. Future studies would need to compare the use of interferon-gamma with a more traditional OIT dose escalation protocol with a slower buildup phase.

Ketotifen

Ketotifen is an H1 anti-histamine and mast cell stabilizer which has been used to treat a variety of allergic diseases including allergic conjunctivitis, asthma, and eosinophilic

gastroenteritis [101–103]. Jagdis et al. aimed to study the effect of concomitant ketotifen dosing with peanut OIT in a randomized single-blind, placebo-controlled pilot study [104]. Six subjects were randomized to ketotifen or placebo (2:1) and pretreated with ketotifen twice daily for 2 weeks, and then started on a traditional OIT regimen with a 1-day modified rush, buildup over 44 and 4 weeks of maintenance. Ketotifen was tapered over the first 4 weeks of OIT buildup. Due to small sample sizes, no statistical comparisons were performed. During the initial escalation day, the ketotifen group experienced a lower reaction rate of 22 versus 67% in the placebo group, as measured by percent of doses associated with symptoms. During the initial 4-week buildup period, both groups experienced similar rates of reactions during dose escalations; however, the rate of OIT-related symptoms at home in the active group was lower (50 versus 82% in the placebo group), with a similar decrease in the rate of gastrointestinal symptoms in particular. After stopping ketotifen, the active group had lower adverse reaction rates in clinic (31 versus 79% in the placebo group) and at home (13 versus 26% in the placebo group). The main side effect of ketotifen was fatigue. This study suggests some potential utility for ketotifen co-administration for adverse effects associated with OIT, but would need to be evaluated in a larger cohort.

Leukotriene Receptor Antagonists

Leukotriene receptor antagonists (LTRAs) act to inhibit the action of pro-inflammatory leukotrienes and have been shown to have efficacy in asthma [105]. Furthermore, there is some conflicting evidence that LTRAs can improve symptoms of eosinophilic esophagitis, though consensus guidelines conclude that there is insufficient evidence to support a role for LTRAs in treatment [106–108]. Takahashi et al. aimed to study the effect of LTRAs using a retrospective case series of five food-allergic children who received LTRAs among 63 children who received OIT. All five failed initial treatment with OIT alone due to recurrent abdominal pain or vomiting, and either could not increase dose escalation or had to stop OIT. Symptoms manifested in these children as early as the first week of buildup to as late as the maintenance period. After 4–7 days of persistent abdominal symptoms, the children were started on LTRAs, after which they were able to proceed with therapy, and were treated for 2–6 months of LTRAs. After reaching the maintenance dose, all children were eventually able to discontinue LTRAs and tolerate OIT. None of the children in the case series or in their clinical cohort of 63 children who received OIT were diagnosed with eosinophilic esophagitis, though of note, none of the five children were evaluated endoscopically.

In summary, there is preliminary evidence for the potential utility of concomitant interferon-gamma, ketotifen, and/or LTRA administration to reduce side effects of OIT, but all of

these findings merit replication in larger adequately powered randomized controlled trials with longer periods of treatment and follow-up.

Conclusion

A number of adjunctive therapies have been developed to improve the safety and efficacy of OIT. Anti-IgE-based therapies appear to be furthest along, with probiotics, Chinese herbal medicines, and TLR-4 agonists currently in early phase clinical trials. Meanwhile, nanoparticles, as well as peptide-based and recombinant vaccines, represent an innovative delivery vehicle for OIT that could improve both efficacy and decrease allergic side effects. A number of factors will need to be evaluated in comparing these treatments, including ability to decrease allergic adverse events, safety of the adjunctive therapies themselves, effect on long-term sustained unresponsiveness, and cost. Further phenotyping of food allergy patients may be necessary to determine which ones respond best to each therapy. However, with so many promising adjunctive therapies on the horizon, it appears likely that clinicians will have a variety of options to optimize the administration of food allergen immunotherapy.

Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals This review article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent This review article does not contain any primary work involving human participants requiring informed consent.

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