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journal homepage: www.elsevier.com/locate/addrOral immunotherapy for food allergy: Clinical and preclinical studies[☆]Mike Kulis, A. Wesley Burks^{*}

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ABSTRACT

Food allergies affect approximately 5% of the U.S. population and have increased in the last decade. In recent years, oral immunotherapy (OIT) has been tested in clinical trials for peanut, milk, and egg allergies in young children. OIT appears to be fairly well tolerated by most subjects and leads to desensitization with a greatly increased threshold of allergen required to induce reactions. Further approaches being investigated in pre-clinical studies in mouse models indicate the potential for using adjuvants, such as TLR9 agonists in combination with OIT; peptide OIT; and non-allergen specific applications such as herbal formulations. Further questions about OIT remain, including the optimal dosing and length of treatment; whether tolerance can be developed; and the exact cellular mechanisms resulting in protection following OIT. With many clinical trials underway across the United States and other countries, and a growing pipeline of preclinical research with translational potential, there is great hope for a widely applicable food allergy treatment.

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1. Introduction

Food allergies affect approximately 4–6% of the population in the U.S. [1]. The most common allergenic foods in the pediatric population are eggs, milk, and peanut; while adults are most often allergic to peanuts, tree nuts, and shell fish [2]. The prevalence of food allergies appears to be increasing. A Center for Disease Control and Prevention study reported an approximately 20% increase in food allergies in U.S. children during a 10 year period [3]. Other studies have demonstrated increases in the prevalence of peanut allergy, indicating a doubling in a ten year period [4–6], while tree nut allergies are reportedly increasing, with a prevalence of 0.2% of children in 1997 to 1.1% in 2008 [7].

Abbreviations: OIT, Oral immunotherapy; SLIT, Sublingual immunotherapy; SCIT, Subcutaneous immunotherapy; TLR, Toll-like receptor; FAHF, Food allergy herbal formula; DBPCFC, Double-blind, placebo-controlled food challenge; Tregs, Regulatory T cells; CoFAR, Consortium of Food Allergy Research.

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The underlying cause of food allergy is an immunologic aberration in the typical oral tolerance response to food antigens. Oral tolerance results in antigen-specific T cell deletion, anergy, or induction of regulatory T cells (Tregs) (Reviewed in detail in [8,9]). Allergy results in a Th2-type immune response, characterized by IL-4, IL-13, IL-5, [10] and possibly other cytokines such as IL-9 [11]. Typically, humans, as well as mice, that are tolerant to orally ingested food antigens have serum that is absent of detectable IgE. Patients with a food allergy have elevated IgE responses against food proteins. These IgE molecules bind to mast cells and basophils which are cross-linked upon antigen encounter, leading to degranulation of these effector cells. A signaling cascade leads to release of allergic mediators, such as histamine, leukotrienes, cytokines, and prostaglandins [12]. Some allergic reactions are very mild in nature and resolve spontaneously, whereas the most severe forms of allergic reactions to foods can trigger anaphylaxis, a life-threatening reaction that can involve several body systems, including the heart, lungs, skin, and gastrointestinal tract [13].

Allergic reactions to food send tens of thousands of patients to the emergency room annually, with most recent estimates at 200,000 visits per year [14]. The current standard of care is strict avoidance of the offending allergens and immediate access to rescue medications, such as epinephrine and antihistamines [15]. The avoidance regimen, while simple, is difficult to achieve and often leads to decreased quality of life by placing a major burden on patients and their families [16]. Natural resolution of IgE-mediated food allergy can occur without any interventional therapy, although it appears to be food specific. Egg and milk allergy are often outgrown by age 10 [17,18], whereas peanut and tree nut allergies are rarely outgrown. Natural resolution of peanut allergy is often quoted at 20% of patients eventually developing tolerance to peanuts without any intervention [19], while tree nut allergy is only resolved in 10% of patients [20]. These data can be daunting to families and patients given a diagnosis of nut allergies, as 80–90% of the patients will have the allergy for life. One reason for the decreased quality of life is that allergic reactions to food can be deadly. Peanuts and tree nuts account for the majority of the life-threatening and fatal reactions [21,22], and may cause severe stress following a diagnosis.

The exact mechanisms involved in natural resolution of food allergies are not completely understood. The allergen-specific IgE will disappear, along with skin prick test reactions, however, little is known regarding the cause [23]. One study showed that the development of milk-specific Tregs in milk allergic subjects ultimately led to tolerance [24], indicating that Tregs may be a driving force in outgrowth of milk allergy, possibly by down-regulating the Th2-skewed responses. Changes in quantity of milk-specific IgE and IgG4 against milk protein epitopes are also observed in those developing tolerance [25] and affinity of IgE binding to milk allergen epitopes may indicate varying phenotypes of allergy [26]. Suppression of basophil activation appears to be involved in tolerance, and may be influenced by the changes in IgE and IgG [27]. Other studies on egg and milk allergy demonstrate that patients with IgE directed primarily against structural epitopes have increased odds of outgrowing the allergy when compared with patients having IgE directed at linear epitopes [28,29]. On the other hand, IgE against peanut allergens appear to be predominantly against linear epitopes and may account for the persistence of peanut allergy [30–32]. A recent publication also demonstrates the potential importance of IgE/IgG4 ratios against the major allergens in egg in predicting varying severities of egg allergy [33]. An observational study in the U.S., conducted by the Consortium of Food Allergy Research (CoFAR) is underway and aims to further elucidate the characteristics and immunologic mechanisms involved in resolution and development of food allergies [34].

In this review, we discuss the state-of-the-art in oral immunotherapy (OIT) for food allergy in pediatric populations. The objective of this review article is to review efficacy of OIT in clinical trials, along with the associated immunologic changes that accompany

OIT. Primary literature on this topic was selected based on study design that would address efficacy, including both open-label and randomized placebo-controlled trials. We also highlight important studies performed in preclinical animal models of food allergy utilizing therapies administered through the oral route.

2. Oral immunotherapy (OIT) for food allergies: clinical studies

While many IgE-mediated allergies are often controlled and treated with subcutaneous immunotherapy (SCIT) [35], food allergies are not amenable to this form of immunotherapy. Attempts were made to treat peanut allergies with SCIT, but side effects were too frequent and severe leading to abandonment of this approach [36]. Some anecdotal reports of efficacy with oral administration of foods had been reported [37–39]. While these studies were not randomized, placebo-controlled trials, the concept was developed that by starting below the eliciting dose, then gradually increasing the dose to much higher levels would prevent reactions to the offending food. Initial studies were designed to determine safety and efficacy in small sample size trials, usually of an open-label design. Follow-up studies, designed after open-label studies indicated that OIT was reasonably safe and efficacious, were designed to include placebo controls, entry food challenges to determine baseline reactivity thresholds, and larger randomized controlled trials with multiple research centers.

2.1. OIT for peanut allergy

Clinical trials utilizing OIT for peanut allergy have included open-label and double-blind placebo-controlled trials to determine the safety and efficacy of OIT. In an open-label study, Jones et al. performed a study in three phases: initial escalation, build-up, and maintenance [40]. The subjects received an initial dose of 100 µg of peanut protein which was escalated to a target of 50 mg on day 1. After the first day, subjects' doses were increased bi-weekly until a maintenance dose of 300 mg, roughly equivalent to the protein in 1 peanut, was reached. Maintenance dosing continued for several months, followed by a 3900 mg challenge, equivalent to approximately 13 peanuts. 27 of 29 subjects were able to tolerate the 3.9 g peanut challenge. The challenge outcomes demonstrated a large increase in threshold dose, termed desensitization, since 93% of subjects tolerated less than 50 mg on the initial escalation day.

Safety was assessed for each phase of the trial, with focuses on severity and medications used to treat symptoms [41]. 26 of 28 subjects had allergic symptoms on day 1, albeit with generally mild symptoms. Only 4 of the 28 subjects studied required epinephrine on day 1. During the build-up phase, approximately 46% of subjects had some form of reactions, but these were all during observed up-dosing conducted on the clinical research units. Home dosing saw only 3.5% of subjects having reactions. The majority of reactions, regardless of the phase in the study were skin, upper respiratory, and gastrointestinal symptoms, while more severe reactions involving the chest were much less frequent.

Immune parameter changes were detected at the immunoglobulin and T cell levels [40]. Peanut-specific IgE was initially increased from 3 to 9 months into the study then were decreased below baseline levels by 12 to 18 months. Peanut-IgG4 was increased by 3 months then stayed elevated throughout the remainder of the study. The IgG4 was found to have function in an inhibition assay of facilitation antigen binding [42] designed to detect blocking of peanut antigen binding to IgE. T cell changes included alterations in cytokines, but more importantly an increase in Tregs. Interestingly, the Tregs increased initially but later returned to baseline levels. The transient increase in Tregs may be a result of homing to other areas of the body or a contraction in the number of Tregs but retention of functional abilities of the remaining Tregs. Further studies investigating the role of Tregs in OIT are needed.

Effector cell changes were also demonstrated. Mast cell responses, as assessed by skin prick testing with peanut proteins, were significantly decreased by as early as 6 months into the study. Further OIT resulted in even greater suppression of the skin prick tests. Basophils, another immune cell with IgE bound to the cell surface through FcεRI, may also play a role in food allergy and represent a potential biomarker to study food allergy responses [43]. Following peanut OIT, basophil activation in whole blood stimulated *ex vivo* with peanut proteins was decreased. As with the skin prick test changes, the basophil responses to peanut were also noted early into the OIT protocol, with changes measured as soon as a few months into the study.

The first double-blind placebo-controlled study of peanut OIT was reported in 2011 [44]. This randomized controlled trial unequivocally demonstrates that peanut OIT is effective in desensitizing peanut allergic subjects as compared to a placebo control group. The study was designed to test the efficacy of OIT after 12 months of therapy. The initial escalation dose was reduced to a target of 6 mg of peanut protein, instead of the 50 mg dose in the open-label trial [40], to limit allergic side-effects on day 1. This approach was successful as only 47% of subjects experienced reactions on day 1, with only 2 of 19 requiring epinephrine. The maintenance doses were increased to 4000 mg from the original open-label protocol's maintenance dose of 300 mg. The study found that 19 of 19 subjects on peanut OIT could tolerate a 5000 mg peanut challenge after 12 months, whereas none of the placebo subjects passed the challenge. The differences in medians tolerated on challenge between OIT and the placebo group was highly significant, with the OIT group tolerating a median of 5000 mg and the placebo group tolerating a median of only 280 mg.

Changes in effector cells were again noted for the OIT group, having significantly decreased responses to skin prick tests and basophil activation assays, which were not detected in the placebo group. Further studies on basophils were conducted in this group of subjects to investigate the possibility of pathway specific anergy induction following constant exposure to peanut antigens *in vivo* [45]. Basophil activation was measured by upregulation of CD63 and CD203c in response to peanut antigens, anti-IgE, and fMLP. The study found decreased activation of basophils following OIT in response to peanut antigen and anti-IgE stimulation, whereas activation with fMLP was not decreased following OIT. These data indicate that peanut OIT induces suppression of basophils through the IgE-receptor pathway, but not in the distinct fMLP receptor pathway. Thus, IgE-receptor pathway suppression may represent an important mechanism of disarming effector cells via OIT.

Immunoglobulin levels and T cell responses were also quantified in this randomized controlled trial. Peanut-IgE was not different at 12 months in either group, however the OIT group did experience increases early on in treatment, similar to the changes reported in the open-label trial. Peanut-specific IgG4 was increased in the OIT group compared to the placebo. Tregs were increased during the OIT period, with associated decreases in Th2-type cytokines, IL-5 and IL-13. The regulatory cytokine TGF-β was increased early but returned to baseline after 9 months, and the Tr1-type cytokine, IL-10 did not change consistently among subjects. It is unclear why there is an apparent disconnect between Treg numbers and presumed regulatory-type cytokines.

An OIT study performed in Germany was designed as a “rush” OIT protocol in which the dosing was designed to increase the subjects up to a dose of 500 mg of peanuts over a maximum of 7 days by increasing the doses 2 to 4 times per day [46]. Only 5 of 22 subjects were able to reach the goal of 500 mg. Importantly, the median DBPCFC after the “rush” phase was not different than entry DBPCFC, thus indicating that desensitization did not occur with the rush OIT approach. The study showed many side effects during the rush phase, and was later modified to allow prolonged build-up. The subjects continued dosing for a median of 7 months, with 2 months of maintenance doses, and were eventually removed from OIT for 14 days before undergoing a

peanut challenge. A median 4-fold increase in DBPCFC after OIT compared to entry DBPCFC was reported. This study on a European cohort confirms similar results from studies in the U.S. [40,44].

A European research group in the U.K. has also published results for peanut OIT. These trials were open-label studies without placebo controls [47,48]. The studies, like the others discussed above, demonstrated efficacy of OIT in peanut allergy. In the larger trial, 86% of subjects experienced reactions during immunotherapy, although none required epinephrine [47]. The researchers conclude that the safety profile of their protocol was good, with generally mild reactions noted. Following the maintenance phase, 14 of 22 subjects tolerated 6.6 g of peanut protein. Importantly, the researchers found a 1000-fold increase in peanut tolerated from baseline challenge to post-OIT challenge. Another study published from the same group focused on 4 subjects and found an increase of ~50-fold in eliciting dose threshold following OIT [48]. These studies in the U.K. again demonstrate the applicability of the OIT protocols to other cohorts outside of the U.S.

2.2. OIT for milk allergy

A study conducted by Johns Hopkins and Duke University researchers was designed to test safety and efficacy of OIT in milk allergy [49]. The study was designed with an entry DBPCFC followed by a 2:1 randomization to receive milk OIT or placebo, respectively. Subjects remained on OIT for approximately 6 months with a maintenance dose of 500 mg of milk protein. Following OIT, subjects underwent a second DBPCFC. The median tolerated dose during entry DBPCFC for the 19 subjects enrolled was 40 mg of protein. Following the dosing period, the milk OIT group tolerated a median of 5140 mg of milk protein (range of 2540–8140 mg) on DBPCFC, compared to the placebo group that tolerated a median of only 40 mg. The large differences between the OIT and placebo groups demonstrate the efficacy of OIT in milk allergy. The researchers also concluded that allergic side-effects were generally acceptable but require further study.

Another study by the same research groups incorporated sublingual immunotherapy (SLIT) with OIT. Subjects all received SLIT with milk proteins, then were randomized to either continue SLIT or cross-over to receive OIT [50]. After 18 months in the study, only 1 of 10 subjects in the SLIT group tolerated the 8 g milk challenge, whereas 14 out of 20 subjects in the groups receiving SLIT then OIT tolerated the 8 g challenge. These outcomes appear to demonstrate that OIT is superior to SLIT in terms of desensitization to milk allergens. It is important to note that OIT was associated with more frequent side effects than SLIT alone, indicating that there is a trade-off with the different routes of administration in terms of efficacy and safety. A similar outcome demonstrating lower efficacy but less frequent side-effects was found with peanut SLIT [51] compared to peanut OIT [52].

A slightly different type of OIT has been based on the finding that approximately 75% of milk allergic patients can tolerate extensively heated milk. For example, the milk in baked goods such as muffins and waffles were tolerated by 77 out of 100 subjects in one study [53]. The fact that a majority of milk allergic patients can tolerate milk in baked food leads to the idea that milk allergy has two phenotypes: one in which heated milk is not tolerated is considered severe, but for the subjects that tolerate baked milk, the allergy is considered mild. The hypothesis of a 2011 publication was that milk allergic subjects tolerating baked milk in their diet would benefit from ingesting it on a regular basis rather than practicing avoidance [54]. This form of OIT is unique in that the subjects are not reactive to the form of food they are ingesting. 65 subjects were tolerant to baked milk and ingested it for a median of 3 years, at which time 39 of these subjects could then tolerate unheated milk. Conversely, 23 subjects that were reactive to baked milk on enrollment saw only 2 become tolerant to unheated milk in the same time period. The authors conclude that ingestion of baked milk appears to be more beneficial than strict avoidance.

2.3. OIT for egg allergy

In the first publication on OIT for egg allergy, 7 subjects were studied in an open-label trial [55]. OIT was initiated with a starting dose of 0.1 mg of egg protein, which was escalated to a max dose of 200 mg on day 1. Subjects underwent bi-weekly build-up dosing until 300 mg was reached for the maintenance dose. 4 subjects out of 7 passed an egg challenge, while all 7 subjects tolerated higher amounts of egg compared to baseline. These subjects were deemed desensitized. The trial was continued with an additional 6 subjects and demonstrated the utility of increasing OIT doses based on egg-specific IgE levels [56]. Subjects ingested an OIT dose with a median of 2400 mg for maintenance. All 6 subjects passed the food challenge. Typical immunologic changes were observed in both egg OIT studies. These changes include decreased mast cell reactivity during skin prick tests, decreased egg-IgE, increased egg-IgG and IgG4 [55]. Th2-type cytokines were decreased and the regulatory-type cytokines, IL-10 and TGF- β were increased [56].

The first double-blind, placebo-controlled trial of egg OIT in subjects with persistent egg allergy has recently been published [57]. The CoFAR group conducted a multicenter clinical trial involving five research centers in the U.S. with a total of 55 children enrolled. The study was designed to test efficacy of OIT after 10 months, then again after 22 months. Desensitization was achieved in 55% of OIT subjects following 10 months of OIT, and 75% were desensitized after 22 months of OIT [57,58]. No placebo subjects were desensitized, indicating that natural tolerance did not develop in this study population of persistent egg allergy. Importantly, OIT subjects that passed the desensitization challenge at 22 months discontinued daily OIT for 4–6 weeks and were then challenged again. Subjects that passed this third challenge (28% of the OIT group) were deemed to have “sustained unresponsiveness” indicating that long-term benefits may be achieved via OIT. As with other OIT studies, immunologic changes include decreased basophil and mast cell responses and decreased egg-specific IgE with increased IgG4.

As described in Section 2.2 for milk allergy, extensively heated (i.e. baked) egg products have been found to be tolerated by a large proportion of patients with egg allergy. It is believed that extensive heating during baking reduces the allergenicity of the antigens, likely by denaturing the proteins and destroying structural, non-linear, epitopes. The denatured protein hypothesis was further evaluated *in vitro* and found that heated egg allergens were more easily digested in simulated gastrointestinal assays and were further shown to be less allergenic in mice [59]. In clinical challenges, 64 of 117 subjects with confirmed egg allergy were able to tolerate baked egg in the form of muffins or waffles [60]. These baked egg tolerant subjects then ingested the baked egg for 12 months. Immunologic changes included decreased skin prick tests to egg, decreased specific IgE, and increased IgG anti-ovomucoid and ovalbumin, two major egg allergens. These immunologic changes appear to indicate a shift towards tolerance. If this form of OIT is found successful it could be a highly efficient way to desensitize subjects with less-severe forms of egg allergy. Since the subjects already tolerate the food in baked form, it poses very minimal risks for side effects. Double-blind, placebo controlled trials with this form of heated allergens are needed to further evaluate this form of OIT.

2.4. Anti-IgE therapy combined with OIT

Anti-IgE therapy is another promising approach to treat many types of allergy, including food allergy. Humanized, monoclonal antibodies directed against human IgE were tested for efficacy in peanut allergy [61]. The trial was a double-blind, placebo controlled trial testing 3 doses of the anti-IgE molecule, TNX-901. Following several months of injections, the final peanut challenges revealed that the highest dose of TNX-901 was effective in approximately 75% of subjects. These subjects could tolerate the equivalent of 9 peanuts following the treatment course. However, this type of therapy would require many injections, probably

once per month, for the rest of the patient's life as the anti-IgE monoclonal antibodies are rapidly cleared *in vivo*. A second study also demonstrated some efficacy for peanut allergy via anti-IgE injections, this time with omalizumab (Xolair), which is already cleared to treat severe asthma [62].

The clinical trial with TNX-901 led to the hypothesis that anti-IgE administered before allergen-specific OIT may decrease side-effects and possibly hasten desensitization in food allergy [63]. The idea being that initial side effects would be stemmed by the anti-IgE therapy, which would then allow for OIT administration leading to rapid desensitization that would be antigen-specific. This concept was demonstrated in SCIT for ragweed allergy [64]. The first such published study for anti-IgE therapy combined with OIT for food allergy was published by a group at Stanford University [65]. 11 milk allergic subjects were enrolled and received 9 weeks of Xolair injections, followed by a rush OIT protocol starting at 0.1 mg of milk protein, which was increased to a maximum tolerated dose of 1000 mg. 9 of 10 subjects reached the 1000 mg dose on day 1, which is much higher than in other OIT studies with target doses of typically less than 50 mg. After an approximately 6 month OIT protocol, in which Xolair was stopped, 9 of 10 subjects passed the final DBPCFC tolerating a cumulative dose of 7250 mg of milk protein. The trial demonstrated that subjects could achieve very high dose levels of milk during the initial escalation. Although the trial was limited by not having a placebo control for the Xolair phase, it leads to the conclusion that anti-IgE therapy may be a strong approach to improve the ability to desensitize patients very quickly with OIT since they can tolerate higher doses immediately following a course of Xolair.

An interim analysis of a similar study conducted for peanut allergy reported similar outcomes [66]. Subjects received 4 months of Xolair treatment, followed by a standard OIT protocol. The subjects in this trial were able to tolerate significantly more peanut during the initial escalation day than a similar group of subjects receiving peanut OIT without Xolair. The median dose tolerated on day 1 for the subjects receiving Xolair was approximately 50-fold higher than the comparison group. Further placebo-controlled studies are needed to definitively demonstrate the utility of Xolair to improve the safety profile and allow higher tolerated doses during initial escalation.

2.5. Desensitization versus tolerance following OIT

Desensitization is defined as an increase in the amount of food antigen required to cause a reaction while consuming daily doses of OIT, whereas tolerance is defined as the ability to consume unrestricted amounts of food antigen following a period of removal from daily OIT dosing. The length of OIT dosing required to cause desensitization is likely much shorter than that required to cause tolerance. Although, at the present time, the two outcomes are not fully understood, it is believed that desensitization occurs in a time frame of weeks to months, whereas tolerance likely requires years of OIT.

In the open-label study for peanut OIT [40], subjects eventually underwent tolerance assessment [67]. The study was designed such that subjects consumed a peanut challenge while on OIT, then were removed from OIT for 4 weeks and underwent another peanut challenge. 19 subjects qualified for this segment of the study and had been on OIT for 33–70 months. All 19 subjects passed the desensitization challenge, whereas 11 of 19 subjects were able to pass the tolerance challenge after removal of OIT for 4 weeks. These results demonstrate that for >50% of subjects, tolerance was achieved. Immunologic assessments were compared between the subjects that became tolerant and those that did not. Significant differences between the tolerant and allergic groups were found in baseline skin prick tests and peanut-IgE. The tolerant subjects had lower baseline peanut-IgE and correspondingly lower skin prick tests. Further analyses are underway to investigate other potential biomarkers of tolerance development, including epitope array analysis for IgE and IgG4 binding. The initial findings conclude that

tolerance is an achievable goal for peanut allergic patients that undergo OIT, although it will likely not develop for all patients. The baseline responses to peanut may be predictive of subjects that can eventually achieve tolerance.

The egg OIT studies described in Section 2.3 also incorporated tolerance challenges. 2 of 4 subjects passed tolerance challenges in the study using a 300 mg maintenance dose [55]. In the trial with increased maintenance dosing and longer time on OIT, all 6 subjects were able to pass a tolerance challenge to egg [56]. The larger, multicenter, placebo-controlled trial found that 11 of 40 subjects were able to achieve tolerance following 22 months of egg OIT with a period of 6–8 weeks of abstaining from egg [58]. The milk OIT study also assessed tolerance and found that 9 of 15 subjects were able to pass a tolerance challenge following removal of milk for up to 6 weeks [50]. However, this study found that some subjects regained allergic reactivity following only 1 week of removal of the OIT dosing.

3. OIT: preclinical studies

Oral tolerance prior to allergic sensitization is well documented. There are potentially different mechanisms, with high-dose tolerance involving deletion of T cells and low-dose tolerance inducing Tregs. This phenomenon is reviewed in [9]. Here we discuss therapeutic approaches following the establishment of experimental allergy. The model of choice for food allergy is the mouse, which generates IgE against allergens, can be sensitized via the oral route, and experience anaphylaxis upon oral or injection challenges to the offending allergen [68]. Typically, mice are monitored for allergic symptoms, such as labored breathing, puffiness of the face, cyanosis, and even death. Body temperatures are also measured to determine the shock response [69]. Allergic mediators, such as histamine, mast cell proteases, and leukotrienes can all be measured post-reaction in serum or plasma samples.

3.1. Antigen-specific approaches

Studies replicating desensitization by OIT in mice have not been readily available. These models would be most useful to further investigate local mucosal, and systemic, immunologic changes induced by OIT. One study presented in abstract form demonstrated that OIT in egg-allergic mice is feasible [70]. Mice were sensitized to egg allergens by oral administration of the allergens in the presence of cholera toxin adjuvant, a known method to induce antigen-specific IgE and Th2-skewed responses [68]. The allergic mice then underwent OIT by feeding either low dose (1 mg) or high dose (50 mg) egg proteins daily for two weeks, followed by a challenge with egg proteins. There was a clear desensitization effect reported for the high dose OIT group, but not in the low dose group [70]. Interestingly, another study by the same group, also reported in abstract form, demonstrated that once the mice stopped receiving the daily egg OIT for a period of 2 weeks, the mice did not pass the subsequent challenge [71]. These studies support the idea that OIT can be effective in mice, however, tolerance was not achieved with only 2 weeks of OIT, and may be investigated further with longer courses of treatment.

Another OIT-type study used a TLR9 ligand, CpG DNA, which mimics bacterial DNA. The study was designed such that mice made allergic to peanuts, received orally either placebo (PBS), peanut, or peanut plus CpG four times over two weeks [72]. The mice receiving peanut plus CpG had the most improved clinical outcomes following peanut challenge. This group had highly increased peanut-IgG2a, decreased IgE, and a Th1-skewed T-cell recall response. The group also showed reduced local inflammation in intestinal tissue samples. The use of innate immune receptors, such as TLRs may be an excellent target to increase the magnitude of anti-allergic responses of OIT in humans. It may also lead to hastened responses, and possibly longer term tolerance induction. A previous study in humans with ragweed allergy showed the effectiveness of TLR9 agonists in treating allergic disease [73].

Peptide immunotherapy has shown promise as an approach to treat allergies [74], though little has been published in the context of food allergy. Peptide immunotherapy given orally in egg allergic mice shows promise [75], and could possibly be adapted to human allergic disease. To design the peptide immunotherapy, the dominant T cell epitopes within a major egg allergen, ovalbumin, were determined in mice. 3 of these peptides were delivered by the oral route for several weeks, followed by a challenge with a large amount of ovalbumin. Mice given peptides had reduced allergic symptoms and histamine release during challenge. The researchers report an increase in FoxP3 and TGF- β from intestinal tissue, indicating a potential modulation of the T cell response by induction of Tregs following the OIT with peptides. A similar finding by the same group was reported for another major allergen of egg, ovomucoid, using orally delivered peptides as a form of OIT [76]. A preliminary study to determine the dominant T cell epitopes of the major peanut allergen, Ara h 2, for human CD4 T cells was conducted and hinted at the potential of using these peptides to treat peanut allergy in humans [77].

In a recent report, daily oral administration of egg white antigen in mice sensitized to egg did not resolve experimentally induced intestinal allergy, as measured by soft stools and inflammation [78]. In fact, it actually appeared to cause sustained inflammation. Interestingly, T cell responses, including proliferation and cytokine secretion were suppressed in mice on daily egg OIT, indicating onset of tolerance. It is unclear if the intestinal inflammation would resolve with prolonged treatment in mice, since in humans the OIT is administered for many months or years. Clearly, further studies with OIT in mice need to be conducted to clarify effective doses, length of treatment, and if there are differences in strains or specific models used.

Finally, an approach using allergen DNA rather than peptides or proteins has led to a further route of investigation. The experimental approach used peanut allergen, Ara h 2, DNA conjugated to chitosan nanoparticles [79]. Mice underwent an oral dosing of the particles with Ara h 2 DNA and were found to have increased secretory-IgA and serum IgG2a anti-Ara h 2. On challenge, the mice showed decreased allergic reactions and histamine release. It appears that immunization with this approach sufficiently reduced allergic responses in a mouse model, although it was not used to treat mice with a pre-established allergic response to peanuts [79]. Further studies are needed to clarify if this approach using an oral delivery system of allergen DNA can protect mice that are already hypersensitive to peanuts.

3.2. Antigen non-specific approaches

In addition to allergen-specific approaches of OIT, non-specific approaches are also of interest. A powerful example is the food allergy herbal formula 2, or FAHF-2, pioneered by researchers at Mount Sinai [80]. This formulation is based on traditional Chinese medicine and is a blend of 9 herbs. The herbal formula administered orally can completely prevent allergic sensitization to peanut in mice when delivered in a prophylactic model [80]. More importantly, it can be used to treat existing peanut allergy in mice [81]. Peanut-allergic mice received 32 mg of the herbal formula twice daily for 7 weeks. Challenges showed immediate and prolonged protection from allergic reactions once the treatment was stopped. It was also demonstrated that after the FAHF-2 was halted and the treatment effect subsided, it could easily be re-established when FAHF-2 was re-introduced, again preventing allergic reactions following peanut challenge. It does not appear to be peanut specific and has efficacy against multiple food allergens in mice with allergy to egg, fish, and peanut [82]. The mechanism appears to work by decreasing IgE, increasing IgG, suppressing Th2-type cytokines [83], and having direct effects on mast cells and basophils [84].

Human clinical trials with FAHF-2 are now underway, with subjects taking 4, 6, or 12 tablets 3 times per day [85]. The initial phase 1 study found the formula to be safe and well tolerated in humans with a variety of food allergies, including peanut, tree nuts, fish, and

shellfish. In a mechanistic assessment, suppression of basophil activation was reported following 6 months of dosing [86]. No significant changes in allergen-specific IgE or IgG4 were detected. Further studies in phase 2 trials are anticipated and may lead to a novel approach of treating multiple food allergies.

Another non-allergen specific approach utilized a fermented soy product to reduce anaphylactic reactions in peanut allergic mice. The concept was to use soy proteins, which are homologous to peanut protein allergens [87], along with the probiotic ImmuBalance. The ImmuBalance product was prepared by fermentation of defatted soybeans with *Aspergillus oryzae* and lactic acid bacteria. Mouse chow was produced with or without the soy product and mice were allowed to feed on the chow for 4 weeks, followed by a challenge with peanut. Reduction in anaphylactic reactions was found in mice fed the ImmuBalance, but not in mice fed standard chow. Peanut-IgE and Th2-type cytokines were decreased in mice fed ImmuBalance. Further research has indicated that soy isoflavones modify dendritic cell responses to peanut [88], and may play a role in the ImmuBalance efficacy. This approach, using homologous proteins and natural products such as isoflavones could be useful for an orally delivered treatment of peanut allergy, and potentially other food allergies, such as tree nuts, in which the allergenic proteins are highly homologous [89].

4. Remaining questions and future studies

Much progress has been made in the last 10 years in developing treatments for food allergies. One of the most promising forms of therapy is OIT, which has repeatedly been demonstrated to have clinical efficacy in trials for peanut, milk, and egg allergies. Studies have been reported from several medical centers around the U.S., as well as in European countries. However, with all the progress, some questions remain. At present, it is still unclear what the optimal maintenance dose should be and whether higher doses are necessarily more effective. The length of time to continue OIT is also not fully understood, although it appears desensitization occurs within months, whereas tolerance may take years to achieve. Trials designed to investigate tolerance should be conducted with subjects being removed from OIT following varying lengths of time, while other trials may investigate how soon desensitization occurs and how long it persists. Biomarkers to determine when the subject is desensitized need to be discovered, such that clinicians can be informed when a subject may be challenged.

Mechanistic studies to-date have primarily focused on mast cells (skin prick tests), basophil responses, immunoglobulin levels, and changes in T cell phenotypes against crude allergen preparations. However, it may be beneficial to focus on specific allergens, i.e. Ara h 2 or 6 in peanut allergy, which have shown value in diagnostics and are the most potent of all the 11 peanut allergens [90–94]. T cell responses have been aimed at cytokine secretion from in vitro cell cultures and enumeration of Tregs. While these studies have been informative, it will be of utmost importance to further investigate allergen-specific cells, possibly through tetramer technology [95]. Other investigation should focus on Treg function in cell suppression assays since numbers might not be correlated with function. Serological responses focused on component-resolved technology and microarrays to specific epitopes will likely reveal important changes induced by OIT. It is quite possible that these more comprehensive, detailed, allergen- and even epitope-specific responses may yield valuable biomarkers, and be useful in predicting subjects that will become tolerant versus those that may only achieve desensitization.

5. Conclusions

Oral immunotherapy for food allergy has been demonstrated to be effective for peanut, milk, and egg allergies. The safety profile of OIT has not been without allergic side-effects, however, it is generally seen as acceptable when performed in the presence of trained

professionals in research environments. Immunologic changes detected following OIT are similar to other forms of allergen immunotherapy that have been used for decades [35,96]. There is no reason to believe that OIT would not work for any and all food allergies, including severe forms of tree nut and shrimp allergies, in addition to the often feared peanut allergy. However, the consensus remains that OIT is not ready for clinical practice [97], and many are eagerly awaiting larger, placebo-controlled clinical trials which the research community agrees are needed. Preclinical studies in mouse models may yield novel treatment approaches through the oral route. As detailed above, herbal formulations, adjuvanted immunotherapy with TLR ligands, and peptide immunotherapy all show promise for translational research. Studies in mouse models may also give us a better understanding of the mechanisms of OIT because of access to tissues that can not easily be obtained in humans. With many clinical trials underway across the U.S. and other countries, and a growing pipeline of research with translational potential, there is real hope for a widely applicable food allergy treatment.

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