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Summary

There is no approved therapy for food allergy. The current standard of care is elimination of the triggering food from the diet and accessibility to epinephrine. Immunotherapy is a promising treatment approach. While desensitization to most foods seems feasible, it remains unclear if a permanent state of tolerance is achievable. The research team at Duke is pioneering immunotherapy for food allergies. Work here has evolved over time from small open-label pilot studies to larger randomized designs. Our data show that immunological changes associated with immunotherapy include reduction in mast cell reactivity, decreased basophil responses, decreased specific-immunoglobulin (Ig)E, increased IgG4 and induction of regulatory T cells. Immunotherapy has generated much excitement in the food allergy community; however, further studies are needed before it is ready for clinical use.

Keywords: immunoglobulin, oral immunotherapy, regulatory T cells, sublingual immunotherapy, tolerance

Introduction

Food allergy is a major public health concern and affects around 6% of young children and 3–4% of adults in westernized societies [1]. Survey data from the Center for Disease Control and Prevention indicate an 18% increase in the prevalence of reported food allergy in US children from 1997 to 2007 [2]. Other allergic conditions such as asthma, allergic rhinitis and atopic dermatitis are also increasing [3,4]. The cause of this increase remains unclear. While the 'hygiene hypothesis' has received significant attention, it does not provide a sufficient immunological explanation for the observed rise in T helper type 2 (Th2)-polarized disease. Conversely, there is evidence that food allergy is caused by a complex interplay between genetic and environmental factors, as well as the food allergens themselves [5]. Some foods are highly allergenic, while others rarely cause sensitization [6,7]. The major allergenic foods that account for about 90% of food allergies in the United States are milk, egg, peanut, tree nuts, soybeans, wheat, fish and shellfish [5].

The current management of food allergy is limited to nutritional counselling, dietary avoidance and treatment of adverse reactions. Accidental ingestion of allergenic foods remains common despite adequate counselling. The inability to completely eliminate the possibility of anaphylaxis causes a great deal of stress to patients and their families. Health-related quality of life is adversely affected, to a greater degree than seen in other chronic diseases of childhood [8].

Approaches currently considered in tackling food allergy include oral, sublingual and epicutaneous immunotherapy with native food allergens and mutated recombinant proteins. Subcutaneous immunotherapy, also known as 'allergy shots', was studied more than 10 years ago, and this type of therapy was able to induce desensitization. Oral immunotherapy and sublingual immunotherapy have been studied more heavily. In this manuscript, we will review our work on immunotherapy.

Mechanisms of reactivity

Food allergy is hypothesized to result from a breakdown in oral tolerance. There is a natural immunoregulatory process that suppresses immunity to antigens experienced via the gastrointestinal tract [9]. Multiple mechanisms of tolerance are likely and include deletion of antigen-specific T cells, induction of anergy in antigen-specific T cells and production of regulatory T cells (T_{regs}) [10,11]. Several factors,

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including route of exposure, antigen properties and genetics, contribute to the development of oral tolerance [10].

With a defect in oral tolerance, these patients develop a Th2-predominant allergen-specific immune response with the production of immunoglobulin (Ig)E antibodies specific to the food allergen. Th2 cells secrete interleukin (IL)-4 and IL-13, driving B cells to produce IgE. Following that, IgE binds to its high-affinity receptor, FcεRI, on mast cells, which line the skin and mucosal tissues. Upon subsequent ingestion of the sensitizing food, an allergic reaction is triggered by cross-linking of IgE receptors on mast cells, leading to degranulation in which these cells release their contents, namely histamine, leukotrienes and prostaglandins. This gives rise to allergic symptoms including urticaria, angioedema, vomiting, diarrhoea, wheezing and anaphylaxis.

Spontaneous clinical tolerance may develop in food allergic patients. However, this varies and depends on the allergen. Food allergy to egg, milk, wheat and soy typically resolve [12–15]. Food allergy to peanut, tree nuts and seafood is often lifelong [16,17].

Immunotherapy in food allergy

Allergen immunotherapy refers to the treatment of a disease by modulating the immune response [18]. Desensitization is the ability to increase the amount of food protein required to induce a clinical reaction, while still on regular immunotherapy. Tolerance is the ability to consume large amounts of the food protein after being off the treatment. In this context, the ultimate goal of immunotherapy for food allergy is to achieve a permanent state of tolerance.

The exact mechanism by which immunotherapy may induce tolerance is not clear. However, immunotherapy appears to alter the T cell responses to the allergen via skewing the Th2 response to a Th1 response and via the induction of T_{regs}. These T_{regs} can be natural (thymus-derived) or inducible (antigen-specific), and both can suppress the immune responses by different mechanisms, including secretion of IL-10 and transforming growth factor (TGF)-β [19]. Both these cytokines have been found to be important in food allergy [11,20–22].

Subcutaneous immune therapy (SCIT)

SCIT has been used for more than a century. It is a successful therapeutic approach for treatment of hay fever and hymenoptera sensitivity [23–25]. Treatment is associated with certain immune changes, including an initial increase in allergen-specific IgE followed by eventual decrease, an increase in allergen-specific IgG4 and an eventual change from a Th2 response to a Th1 response.

In a study utilizing aqueous peanut extracts, three treated subjects displayed a 67–100% decrease in symptoms induced by double-blind, placebo-controlled peanut challenge [26]. These subjects also demonstrated a 2–5-log reduction in end-point skin prick test reactivity to peanut, while one placebo-treated subject had no change in these parameters. Following a pharmacy error, one placebo-treated subject died of anaphylaxis following accidental administration of peanut extract, resulting in termination of the study. This event highlighted the serious risks of food SCIT.

In a follow-up study from the same medical centre, six subjects were treated with a maintenance dose of 0·5 ml of 1 : 100 wt/vol peanut extract and six were followed as an untreated control group for 12 months [27]. At the end of 12 months, the six treated subjects demonstrated increased tolerance to double-blind, placebo-controlled peanut challenge and decreased skin prick test reactivity to peanut. The untreated controls had no improvement in those parameters. Significant adverse events were recorded in the treatment group during both rush and maintenance immunotherapy, suggesting that this may be an unfavourable treatment modality.

Oral immunotherapy (OIT)

OIT involves the regular and gradual administration of small amounts of allergen by oral ingestion to first induce desensitization and then, hopefully with time, induce tolerance to the allergen. Patients generally ingest a mixture of the allergen protein powder that is mixed with a vehicle (such as apple sauce). This treatment is performed in a monitored setting where the dose of allergen is increased gradually up to a target dose. Following this, most dosing (i.e. maintenance dosing) is conducted at home.

There have been random reports in the literature on the use of OIT for food allergy within the past century. Successful OIT in a boy with egg-induced anaphylaxis was first reported in 1908 by Schofield [28]. Early uncontrolled studies from the 1980s and 1990s provided evidence that successful desensitization to milk, egg, fish and fruit is possible with standardized OIT protocols [29,30]. Further work showed that clinical desensitization using OIT was accompanied by a decrease in allergen-specific IgE and an increase in allergen-specific IgG4 [31,32]. Because these immunological findings are similar to those seen in traditional desensitizing treatments for respiratory allergies, it was thought that defects in oral tolerance causing food allergy could be overcome by OIT. This paved the way to an increase in OIT research. At that point, a significant paradigm shift in the treatment of food allergy was on the way: active therapy with regular exposure to the allergen instead of strict avoidance.

Our initial goal for OIT was to bring subjects safely to a maintenance dose equivalent to a protein amount that is present in a bite of the allergenic food. This should confer protection in the case of accidental ingestion of the allergenic food. In the case of peanut and egg, this dose was determined to be 300 mg. This is equivalent to one peanut or a few bites of egg in a baked cake.

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Egg OIT

A pilot study of OIT in children by our group demonstrated that OIT could be used safely in children with non-anaphylactic egg allergy [33]. The goal of the OIT protocol was the ingestion of a maintenance egg protein dose of 300 mg daily, with the expectation that this dose would both protect subjects from a reaction in case of accidental ingestion and may induce oral tolerance to egg. This 24-month protocol involved modified rush, build-up and maintenance phases. Two double-blind, placebo-controlled food challenges were conducted to determine whether subjects were desensitized or tolerant. Seven subjects completed the protocol. Four subjects passed the initial desensitization challenge, and all subjects tolerated significantly more egg protein than they did at baseline. Subjects who passed the first challenge underwent a second food challenge after a 3–4-month interval without OIT. Two of the four subjects passed this tolerance challenge. Egg-specific IgG concentrations increased in the subjects significantly from baseline. Egg-specific IgE concentrations decreased from baseline, although this decrease was not statistically significant. A key limitation of this study was that it lacked a control group.

In a follow-up study, six more subjects were studied to investigate the effects of higher maintenance dosing [34]. These subjects underwent an OIT protocol consisting of three phases: initial day escalation, build-up and maintenance. Once a maintenance dose of 300 mg was reached, it was continued for 4 months and serum-specific IgE egg white was measured. If the IgE egg white level was greater than 2 kU/l, the patient underwent an open oral food challenge to assess desensitization and the dose was increased according to the highest tolerated dose (by a maximum of 300 mg). This new dose was continued for 4 months and the IgE egg white level was rechecked. For as long as the IgE egg white level remained greater than 2 kU/l, a 4-month cycle of reassessment and 600 mg dose increase continued, to a maximum of 3600 mg/day. Patients were evaluated every 4 months during this maintenance dosing. Whenever the IgE egg white level was less than 2 kU/l, OIT was stopped and a double-blind, placebo-controlled food challenge was performed the following day. All these subjects passed the desensitization challenge and were taken off OIT for 4 weeks. Following that, all six subjects passed the tolerance challenge. These subjects are no longer egg allergic and continue to eat egg in their diet without any problems. This study highlighted the possibility that higher and individualized OIT dosing as well as a prolonged OIT duration may be key in the achieving tolerance.

Our data from egg OIT is not surprising and compares to earlier OIT studies with milk, egg and fish, where there was a decrease in serum-specific IgE and an increase in serum-specific IgG4 [32]. Both egg white and ovamucoid IgE levels decreased significantly during OIT, and so did the median wheal diameter on egg white skin testing. The egg white-specific IgG4 was increased significantly. Trends in egg-induced cytokine production suggested a shift from a Th2 to a Th1 phenotype with transient production of the immunoregulatory cytokines IL-10 and TGF-β, but this did not reach statistical significance possibly because of the limited number of subjects [34].

Milk OIT

The research group at Duke was involved in a trial of milk OIT in collaboration with the group at Johns Hopkins [35]. This was a randomized, double-blind, placebo-controlled trial with an entry challenge to milk. Twenty children were randomized to milk or placebo OIT and 19 completed this study. Dosing included three phases: the build-up day (final dose, 50 mg), daily doses with eight weekly in-office dose increases to a maximum of 500 mg, and continued daily maintenance doses for 3–4 months. The median milk threshold dose required to induce reactions at the beginning of the study was 40 mg. The median challenge dose post-OIT was increased to 5100 mg in the active treatment group. All patients in the placebo treatment group still reacted at 40 mg. Other than showing that milk desensitization is safe and efficacious, this study showed that milk-specific IgG4 increased during milk OIT. However, milk-specific IgE levels remained unchanged. A possible explanation for this was that the short duration of treatment in that study was not long enough to elicit a decrease in milk-specific IgE levels.

Peanut OIT

Until 2009, the literature on peanut immunotherapy consisted mainly of random case reports, with no real trials exploring the clinical response and immunological effects of peanut OIT. In contrast to egg allergy, in most cases peanut allergy persists. In a small study of peanut OIT, Clark *et al.* reported a significant increase in the amount of peanuts that four patients could tolerate following OIT [36].

In a larger open-label study, children with peanut allergy underwent an OIT protocol including initial day escalation, build-up and maintenance phases [37]. Subjects were brought to a 300 mg peanut protein daily maintenance dose over several months. They were challenged following 4–22 months of maintenance dosing to assess desensitization. At this challenge, 27 of 29 (93%) children were able to tolerate 3·9 g peanut protein (equivalent to 16 peanuts). Associated immunological changes included smaller titrated skin prick tests and basophil hyporesponsiveness. Peanut-specific IgE decreased, whereas peanut-specific IgG4 increased. Serum factors (presumably peanut IgG4) inhibited IgE-peanut complex formation in an IgE-facilitated allergen binding assay. Secretion of IL-10, IL-5, interferon (IFN)-γ and tumour necrosis factor (TNF)-α from peripheral blood mononuclear cells (PBMCs) increased over a period of 6–12 months.

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As T_{regs} are thought to play a role in tolerance, peanut-specific forkhead box protein 3 (FoxP3)-positive regulatory T cell levels were thought to change during peanut OIT. Indeed, those increased until 12 months and decreased thereafter. This transient increase in T_{regs} may drive the suppression of the Th2 response. Finally, determination of differential expression of genes in subject samples before and after OIT showed down-regulation of genes involved in apoptotic pathways with OIT.

Peanut OIT was found to be relatively safe in this cohort [38]. Although allergic side effects occurred, they were mainly mild. Significant allergic symptoms were more likely to occur during the initial escalation day, when subjects were in a closely monitored setting, rather than other phases of the study.

We have noted five patterns associated with a tendency for reactions to a previously tolerated dose of peanut OIT. These were: concurrent illness, suboptimally controlled asthma, dose administration on an empty stomach, physical exertion after dosing and dosing during menses [39].

To address tolerance, eight subjects in the open-label OIT study whose peanut IgE levels were reduced to less than 15 kU/l underwent an oral food challenge 4 weeks after stopping OIT [40]. All eight subjects passed the oral food challenge (OFC) demonstrating tolerance to peanut. We continue to follow these subjects.

A randomized double-blind, placebo-controlled trial of peanut OIT was conducted to address desensitization and concurrent immune modulation [41]. Twenty-eight subjects were enrolled in the study. The active treatment group consisted of 19 subjects and the placebo group consisted of nine subjects. Three peanut OIT subjects withdrew early in the study because of allergic side effects. All 16 peanut OIT subjects in the active treatment group passed the OFC and ingested the maximum cumulative dose of 5000 mg (approximately 20 peanuts). None of the nine placebo subjects did. The placebo-treated group ingested a median cumulative dose of 280 mg, before stopping the OFC because of allergic symptoms.

In active subjects, but not controls, peanut-specific IgE increased initially with OIT, but was not significantly different from baseline levels at the OFC. Peanut-specific IgG and IgG4 increased after treatment and continued to rise throughout the first year. Titrated SPT size decreased by the time of OFC. Peanut OIT induced a decrease in IL-5 and IL-13 production, suggesting a shift from a Th2 phenotype to a Th1 phenotype. Peanut OIT subjects had an increased ratio of $FoxP3^{hi}$: $FoxP3^{intermediate}$ $CD4^{+}CD25^{+}$ T cells at OFC, suggesting that the induction of T_{regs} would suppress the allergic immune response.

Sublingual immunotherapy (SLIT)

SLIT involves placing drops of allergen extract under the tongue, then swallowing the liquid. It has been shown to improve clinical symptoms in a variety of IgE-mediated respiratory diseases, including asthma and rhinoconjunctivitis [42,43]. A hypothesized advantage of this modality is the direct absorbance into the blood stream with avoidance of first-pass metabolism in the liver. It is thought that SLIT works by allergen interaction with Langerhan's cells in the oral mucosa, which are protolerogenic. This leads to down-regulation of the allergic response. A few studies using SLIT for food allergy have been reported. A case report described a 29-year-old with a history of several episodes of anaphylaxis after kiwi fruit consumption who underwent successful SLIT with maintenance of tolerance even after cessation of kiwi fruit intake [44].

One of the first double-blind, placebo-controlled trials using SLIT for food allergy was performed by Enrique and colleagues [45]. In this study, 23 patients with hazelnut allergy were enrolled and divided into two groups (active treatment and placebo). Mean hazelnut quantity causing objective symptoms increased from 2·29 g to 11·56 g in the active treatment group *versus* 3·49–4·14 g in the placebo group. Almost 50% of patients who underwent hazelnut SLIT reached the highest dose (20 g), but only 9% in the placebo group did. There was an increase in IgG4 and IL-10 levels after immunotherapy in the active treatment group only.

Recently, our group has published the results of a randomized, double-blind, placebo-controlled trial of SLIT for peanut allergy in children [46]. Eighteen subjects aged 1–11 years completed the study, with 11 randomized to the active treatment group and seven to the placebo group. SLIT was administered daily with peanut extract dissolved in phenol and glycerinated saline in the active treatment group. The placebo treatment group received glycerinated saline solution with phenol and caramel colouring. SLIT began at 250 ng of peanut protein, which was up-dosed to a maintenance dose of 2 mg peanut protein. Dosing side effects were primarily oropharyngeal and uncommonly required treatment. During the double-blind, placebo-controlled food challenge, the treatment group ingested a median of 1710 mg peanut protein *versus* 85 mg in the placebo group. Hence, the treatment group was able to consume 20 times more peanut protein on average than the placebo group.

SLIT was associated with a decrease in skin prick test wheal size and basophil responsiveness. Peanut-specific IgE levels increased over the initial 4 months and then decreased steadily over the remaining 8 months, whereas peanut-specific IgG4 levels increased during the 12 months. After treatment, IL-5 levels were significantly lower in the active treatment group compared with those in the placebo group. IL-13 in the active treatment group also decreased, but this was not statistically significant. Lastly, an increased percentage of T_{regs} was seen in the active treatment group, but this was not statistically significant when compared with the placebo.

Symptoms were reported with 11·5% of peanut doses *versus* 8·6% of placebo doses. The majority of reactions were transient oropharyngeal itching (in the peanut SLIT

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Table 1. Summary of our work on immunotherapy for food allergy.

Year	Author	Food	Type	Age (years)	Blinded	Immunoglobulin changes
2007	Buchanan *et al* [33]	Egg	OIT	1–7	No	Decrease in IgE (not statistically significant); Increase in IgG
2008	Skripak *et al* [35]	Milk	OIT	6–17	Yes	No change in IgE; increase in IgG4
2009	Jones *et al* [37]	Peanut	OIT	1–16	No	Decrease in IgE; increase in IgG4
2011	Vickery *et al* [34]	Egg	OIT	1–16	n.a.	Decrease in IgE; increase in IgG4
2011	Varshney *et al* [42]	Peanut	OIT	1–16	Yes	No change in IgE; increase IgG4
2011	Kim *et al* [47]	Peanut	SLIT	1–11	Yes	Decrease in IgE; increase in IgG4

Ig, immunoglobulin; OIT, oral immunotherapy; n.a., not available; SLIT, sublingual immunotherapy.

group) and pruritis (in the placebo group). Fewer than 0·3% of peanut home doses required treatment with an anti-histamine. One peanut home dose required treatment with albuterol. No placebo doses required anti-histamine or albuterol treatment. Epinephrine was not required for any doses during the study. Symptoms requiring treatment included lip swelling, throat itching, finger swelling, pruritis and wheezing (one episode in one patient).

Ongoing studies at Duke

Ongoing studies for food allergy at Duke are multiple. We continue to look for immunological parameters that may predict the likelihood of immune tolerance in food allergy patients. We have started a pilot study using Omalizumab (anti-IgE) in conjunction with peanut OIT (ClinicalTrials.Gov #NCT00932282). This study will tell us whether anti-IgE therapy can reduce side effects and allow for an accelerated build-up phase. Another ongoing trial is the DEVIL study (Determining the efficacy and value of Immunotherapy on the Likelihood of Peanut Tolerance; ClinicalTrials.Gov # NCT00932828). While recent studies suggest the importance of early oral exposure in tolerance induction, we are examining whether early treatment of peanut allergic infants with peanut immunotherapy may prove beneficial.

Our research team at Duke is also part of CoFAR (Consortium of Food Allergy Research), which has multiple ongoing multi-site trials at Mount Sinai School of Medicine, National Jewish Medical Center, University of Arkansas Medical Sciences and Johns Hopkins School of Medicine.

Summary

Table 1 summarizes our work on Immunotherapy for Food Allergy at Duke. There is no approved treatment for food allergy, and avoidance remains the standard of care. A large body of evidence has accumulated showing the successful induction of desensitization by OIT and more recently by SLIT. Immunological changes associated with OIT and SLIT include reduction in mast cell reactivity, decreased basophil responses, decreased specific-IgE, increased IgG4 and induction of T_{regs}. Although the potential for the development of long-term immunological tolerance remains to be identified, OIT and SLIT offer promising hope and optimism for food allergy patients and their families.

Disclosure

Dr. Burks has received grant support from the Food Allergy and Anaphylaxis Network, the National Institutes of Health, and the Wallace Research Foundation; has served on boards for the American Academy of Allergy Asthma and Immunology, Food Allergy and Anaphylaxis Network, US Food and Drug Administration, *Journal of Allergy and Clinical Immunology*, and National Institutes of Health HAI; and has received other support from the Food Allergy Research Project Fund, Food Allergy and Anaphylaxis Network, National Institutes of Health, and the Wallace Research Foundation.

Dr. Burks has served as a consultant for ActoGeniX NV, Dannon Co. Probiotics, Intellijject, McNeil Nutritionals, Novartis, Nutricia, Pfizer, and Schering-Plough Corp. and owns stock/stock options in Allertein Therapeutics and MastCell.

Dr. Mousallem reported no potential conflicts of interest relevant to this article.

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