Current Allergy and Asthma Reports (2019) 19: 15
https://doi.org/10.1007/s11882-019-0846-6

IMMUNOTHERAPY AND IMMUNOMODULATORS (B VICKERY, SECTION EDITOR)

Hypoallergenic Proteins for the Treatment of Food Allergy

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Published online: 22 February 2019

Abstract

Purpose of Review Food allergy is a growing health problem worldwide that impacts millions of individuals. Current treatment options are limited and strict dietary avoidance remains the standard of care. Immunotherapy using whole, native allergens is under active clinical investigation but harbors the risk of severe side effects including anaphylaxis. Newer food-specific therapies with hypoallergenic proteins may potentially offer safer treatment alternatives, and this review seeks to investigate the evidence supporting the use of these modalities.

Recent Findings The utilization of different methods to alter allergen structure and IgE binding leads to reduced allergenicity and decreases the risk for systemic reactions, making the use of potential therapies including extensively heated egg/milk, peptide immunotherapy, recombinant allergen immunotherapy, and DNA vaccines safe and possibly efficacious forms of treatment in food allergy. However, for the majority of these treatment modalities, limited data currently exists looking at the safety and efficacy in human subjects with food allergy.

Summary This review provides a comprehensive overview of the current evidence examining the safety and efficacy of hypoallergenic proteins in the treatment of food allergies.

Keywords Food allergy · Vaccines · Immunotherapy · Recombinant allergens

Introduction

The prevalence of IgE-mediated food allergy is a rapidly growing health problem affecting millions of adults and children alike, worldwide. It is estimated that 15 million individuals, of which 5.9 million are children under the age of 18, are impacted by food allergy in the USA alone. Epidemiologic studies suggest an increasing prevalence over time that coincides with an increase in other atopic diseases, like eczema, over the last two decades [1–3]. The risk with IgE-mediated food allergies involves possible life-threatening hypersensitivity reactions, and treatment options are exceedingly limited. At the present time, there are no FDA-approved treatments for food allergy, although significant resources are being directed towards identifying potential treatment modalities [4, 5]. Currently, standard therapy involves extensive patient education, strict avoidance of the offending food(s), and immediate treatment of allergic reactions due to accidental exposures, using self-administered epinephrine if needed. Persistent fear of accidental ingestion and anaphylaxis can be very anxiety-provoking for patients and families and impact the quality of life [6, 7]. There can also be a significant financial burden on patients and society for treatment of reactions as strict food avoidance can be difficult to maintain [8].

To help decrease reactivity to culprit foods, many therapies are currently under investigation for the treatment of food allergy, the most commonly studied of which is allergen-specific immunotherapy using whole, native allergens administered via three main routes: oral (OIT), sublingual (SLIT), or epicutaneous route (EPIT). Clinical trials have shown promise with many subjects achieving a level of desensitization and a smaller subset achieving sustained unresponsiveness, defined as an increased threshold of reactivity to an allergen even during a period off therapy. But the use of whole, native allergen extracts possess several disadvantages including the risk of inducing IgE-mediated reactions, so newer food-specific

This article is part of the Topical Collection on *Immunotherapy and Immunomodulators*

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therapies utilizing hypoallergenic proteins have been evaluated as potentially safer treatment alternatives in the treatment and prevention of food allergies. This review will explore the scientific mechanisms and clinical studies investigating the use of these newer therapeutic modalities, including baked egg and milk, peptide-based immunotherapy, recombinant allergen immunotherapy, and DNA-based vaccines (Table 1).

Pathophysiology of Food Allergy

IgE-mediated food hypersensitivity accounts for the majority of allergic reactions to foods. The body is first exposed to an ingested food allergen at the mucosa of the gastrointestinal tract. Proteins are taken across the epithelial barrier and presented to antigen-presenting cells (APCs), which process the proteins into smaller peptide fragments and present them on the cell surface via MHC Class II molecules. These peptides are recognized by naive T-helper cells, and via interactions at the MHC Class II molecule/T cell receptor interface, the naïve T-helper cells differentiate into specific Th2 cells. Cytokines, including IL-4, IL-5, and IL-13, are released by these Th2 cells, which promotes class switching in plasma B cells and production of food-specific IgE. This process is called sensitization. These IgE molecules are released into systemic circulation where some remain bound to the surface of mast cells and basophils, waiting for re-exposure to the same allergen. When re-exposure to allergen occurs, adjacent IgE molecules on the cell surface become cross-linked, leading to activation of mast cells and basophils and release of inflammatory mediators including histamine, leukotrienes, and prostaglandins. It is the action of these chemical mediators that promote the development of clinical manifestations of allergic reactions including urticaria, pruritis, angioedema, and anaphylaxis [9, 10].

Baked Milk and Egg

Extensively heated milk and egg have been considered for use in treatment of food allergies since high heat disrupts conformational epitopes in allergenic proteins, leading to decreased IgE binding and risk for clinical reactivity. The interaction between heated milk and egg proteins with food matrices can also decrease their accessibility to digestion and slow their absorption. It is estimated that up to 80% of individuals with milk or egg allergy can tolerate the baked forms of these foods, indicating the hypoallergenic nature of this form of the antigens. There is evidence to suggest that regular ingestion of baked milk or egg can lead to immunologic changes similar to those seen in oral immunotherapy trials and can promote the development of clinical tolerance [11, 12]. Thus, extensively heated forms of milk and egg could be theoretically utilized as a form of modified OIT that can lead to the development of desensitization to foods containing milk and egg that are not as extensively heated. But for those individuals who are not able to safely ingest baked milk or egg products at baseline, there are limited studies that actually outline a desensitization protocol to safely escalate the dose of these foods over time.

In one study, baked milk OIT in baked milk reactive subjects who had failed unheated milk OIT was attempted. Patients were administered baked milk (heated at 180 °C for 30 min) at doses less than their baseline eliciting dose 50% dose increases performed monthly until 1.3 g per day of baked milk protein was reached. Only three out of 14 patients (21%) reached the goal dose of 1.3 g daily after 1 year of OIT. Eight patients ultimately failed OIT because of IgE-mediated systemic reactions, including two patients experiencing anaphylaxis requiring epinephrine administration. A modest but significant increase in the reactivity threshold to unheated milk was noted in those individuals who were able to complete 12 months of therapy. Study authors concluded that these results supported prior observations that individuals with a more severe phenotype of milk allergy are more likely to react to baked milk and that caution must be exercised and a further study conducted on the use of baked milk as a form of immunotherapy [13].

In contrast, a study on the efficacy and safety of utilizing baked egg as oral immunotherapy enrolled 15 subjects who had evidence of reactivity to baked egg at the time of screening. Patients were started on an initial dose of 125 μg of egg protein, which was increased daily over 60 days to a target maximum of 6.25 g of egg protein daily. Once this target dose was reached, an open egg challenge using a boiled egg was performed to assess for desensitization. Eight subjects (53%)

Table 1 Scientific mechanisms and clinical studies investigating the use of newer therapeutic modalities

Methods to reduce IgE reactivity in hypoallergenic proteins	
Baked egg and milk	High heat disrupts conformational epitopes, leading to decreased IgE binding
Peptide immunotherapy	Use of short, synthetic T cell epitopes eliminates the ability to cross-link IgE
Recombinant allergens	Destruction of conformational epitopes via the use of recombinant protein fragments, denaturation of recombinant wild-type allergens (folding variants), mosaics, or mutants leads to decreased IgE binding
DNA-based vaccines	Use of plasmid DNA vectors, rather than proteins, to induce immunomodulatory effects

were able to complete the OIT treatment regimen and tolerate the open egg challenge, although four of these subjects did need between 80 and 270 days to complete the program [14].

Giavi et al. investigated the use of a low allergenic hydrolyzed egg preparation (HydE) as oral immunotherapy in egg-allergic children. Prior studies had confirmed that HydE could induce desensitization in mice while maintaining low allergenicity [15, 16]. Twenty-nine subjects were randomly assigned to treatment with either a daily dose of 9 g of HydE or placebo for 6 months. Subjects given HydE tolerated an oral challenge using the full maintenance dose on day 1 and subsequently continued that dose without needing to advance through a buildup phase. At the end of 6 months, an oral food challenge with one boiled egg was performed but the difference in pass rate between the treatment and placebo groups was not statistically significant (36% of the HydE group versus 21% of the placebo group). However, significant changes were noted in specific IgG4 levels and basophil activation between treatment and placebo groups. Study authors concluded that there was evidence to support safety and an immunomodulatory effect in the use of HydE, but that clinical efficacy still needed to be confirmed [17].

Peptide-Based Immunotherapy

Background

As mentioned previously, a disadvantage of immunotherapies that utilize whole, native allergens in the treatment of food allergy is the risk of adverse reactions, including anaphylaxis, due to the intact allergen retaining multivalent B cell epitopes and IgE cross-linking potential. By utilizing soluble peptides made of short, synthetic T cell epitopes, one theoretically removes the ability to cross-link IgE on mast cells or basophils, thus eliminating the risk for immediate-type allergic reactions. T cell epitopes, which are the specific parts of an allergen that are immunodominant, are still able to stimulate T cell responses and downregulate the Th2 pathway, which modulates the allergic response. Peptide-based food allergy vaccination is a proposed method of treatment of food allergies that is still under early investigation but may offer an improved safety profile compared with classic immunotherapy techniques while retaining overall efficacy [18].

The peptide vaccine manufacturing process begins with the identification of all potential T cell epitopes within an allergen. Epitope mapping requires sequencing of an allergen and isolation of allergen-specific T cell lines from large donor cohorts, a process which has been made more efficient through recent advances in biotechnology. These T cell lines are screened for reactivity against synthetic peptides sequenced after the target allergen. Once T cell reactive sites on peptides are identified, their precise sequences are evaluated for their ability to stimulate T cells, and those with the strongest immunogenic potential are selected for immunotherapy. Peptide modification is sometimes required to improve solubility and stability prior to manufacturing the final product. The efficacy of peptide immunotherapy has been demonstrated in some studies of perennial and bee venom allergies [18–21]; however, a large double-blind multicenter phase III trial performed by biopharmaceutical company Circassia looking at the use of Fel d 1 allergen peptides in cat allergic individuals failed to demonstrate a difference in symptom scores between treatment and placebo groups [22]. The use of peptide immunotherapy in food allergy has been less widely explored. A Phase I Australian trial investigating the safety and tolerability of PVX108 (a solution comprised of T cell epitopes of peanut allergens Ara h 1 and Ara h 2) in peanut-allergic adults is currently underway with results still pending (ACTRN12617000692336).

Egg Allergy

Hen's eggs are one of the most common foods implicated in food allergy worldwide, with an estimated 2% of infants and children being affected. The major allergens identified in eggs include ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), lysozyme (Gal d 4), and albumin (Gal d 5) with the majority of these allergens (Gal d 1–4) being present in egg white rather than egg yolk. Out of these five allergens, ovalbumin (Gal d 2) is the most abundant protein, making up about 54% of the total protein content in egg whites [23].

Yang et al. investigated the therapeutic potential of peptide immunotherapy using 15 amino acid synthetic peptides manufactured from three immunodominant T cell epitope sequences that were identified in a BALB/c mouse model of allergy to ovalbumin (Gal d 2). A peptide sequence length of less than 20 amino acids was selected in order to optimize MHC Class II binding while minimizing the potential to cross-link IgE [24]. In this study, mice were sensitized to ovalbumin with repeated oral feedings after following an egg-free diet. These mice were then stratified into either a placebo group, which was given a PBS solution devoid of peptides, or four treatment groups which were given single synthetic peptide doses or a mixture containing all three peptides. Following a 3-week treatment period of subcutaneous immunotherapy injections that were administered three times a week, the mice underwent oral challenges with high doses of ovalbumin to trigger anaphylaxis. Mice treated with a solution of multiple-epitope-containing peptides achieved lower anaphylaxis scores, lower serum histamine levels, and lower OVA-specific IgE levels compared to mice in either the single-peptide or placebo-treated groups. The co-administration of three OVA T cell epitopes also produced significantly higher mRNA expression of FoxP3 and TGF-β

in intestinal tissues. FoxP3 expressing T cells are known for their inhibitory effects on Th2-allergic responses while TGF-β inhibits effector T cells and acts as a regulator in the induction of FoxP3 expression in regulatory T cells. This suggests a potential modulatory effect of the T cell response. Interestingly, mixed responses were seen in groups treated with single peptides compared to a multiple-epitope-containing solution. Peptide SL-15 actually exacerbated the allergic response with higher serum histamine, OVA-specific IgE levels, and anaphylactic scores whereas treatment with AI-15 or AG-15 attenuated the allergic response. Discordance was also seen when looking at mRNA expression of FOXP3 and TGF-beta in mice treated with single peptides. Prior studies have suggested that immune reactivity can differ greatly depending on the epitope region involved due to differences in MHC-binding, which can promote either a T_H2- or T_H1-skewed response, so this could explain the discrepancies in the study data [25]. The authors concluded that ovalbumin peptide immunotherapy utilizing the administration of multiple T cell epitopes led to suppressive effects in egg allergy in a mouse model that may be used to better understand mechanisms of peptide immunotherapy in egg allergy in humans [26].

In order to mitigate any risk of systemic reactions associated with subcutaneous injections, another study by Rupa et al. investigated the use of T cell epitope targeted oral immunotherapy using immunodominant epitopes of ovomucoid (Gal d 1) in a BALB/c mouse model of egg allergy. Amino acids 157–171 of T cell epitopes in the ovomucoid protein were chosen as the template in the design of two synthetic peptides utilized in this study: single peptide (SP, amino acids 157–171) or multiple peptide (MP, amino acids $[157–171]_3$) [27]. After following a strict egg-free diet, BALB/c mice were orally sensitized to ovomucoid and randomized to either a placebo group or one of two treatment groups. The placebo group was treated with a phosphate-buffered saline solution two times weekly for 4 weeks. The treatment group received either 4 weeks of single-peptide or multiple-peptide treatments. All mice then underwent an oral challenge with high-dose ovomucoid and outcomes were measured with clinical signs of hypersensitivity, serum histamine, antibody activity, and cytokine levels. Compared to mice in the control group, mice in both treatment groups had decreased frequency of clinical signs following oral challenge. Specific ovomucoid IgE was decreased and IgA was increased in both treatment groups. SP-treated mice had decreased histamine and IgG1 levels and increased IgG2 levels compared to both placebo and MP treated groups. IL-4 was significantly reduced in both groups whereas IL-12p70 and IL-10 were increased in SP-treated mice. IFN-ɣ, IL-17, and TGF-β levels did not differ significantly between groups. There was a significant increase in the percentage of CD4+FOXP3+ and CD4+CD25+ cells in the spleen in the SP group. Authors concluded that oral immunotherapy using synthetic peptides of T cell epitopes of ovomucoid was a potentially viable and safe treatment option in egg allergy and validated the potential usefulness of ovomucoid as an immune modulator [28].

Milk Allergy

Similar to hen eggs, cow's milk is one of the most common food allergens and milk allergy affects up to 2.5% of the world's children. The vast majority of individuals with milk allergy do outgrow the condition by adolescence. Caseins (Bos d 8), of which αS1-casein is the most abundant, make up 80% of the total protein content in milk. Whey proteins make up the remaining 20% with α-lactalbumin (Bos d 4) and β-lactoglobulin (Bos d 5) being the most prominent members of this group. An aspect of milk allergy that makes it potentially more complicated and difficult to treat is that allergic individuals are usually sensitized to multiple proteins, with the most frequently identified allergens being caseins, α-lactalbumin, and β-lactoglobulin [29]. IgE and T cell epitopes have been identified in milk proteins including αS1-casein and β-lactoglobulin by prior studies, with some data suggesting differences in epitope binding in patients with transient versus persistent cow's milk allergy [30–33]. Research investigating whether synthetic peptides manufactured from these epitopes might be useful in the treatment of milk allergy is still lacking.

Peanut Allergy

Peanut allergy is a life-threatening and oftentimes lifelong condition. Even the best attempts at prevention of allergic reactions with strict avoidance strategies are met with failure due to the pervasive use of peanut in food preparation [34]. Attempts at treating peanut allergy with subcutaneous injections of peanut extract showed that a level of desensitization could be obtained in some patients but repeated systemic reactions were common, prompting researchers to conclude that a modified form of peanut extract would need to be developed to improve safety and tolerability [35, 36]. Ara h 1 and Ara h 2 are major peanut allergens that have been identified as potential targets for peptide immunotherapy, and research into the identification of T cell epitopes in these allergens is crucial to isolating targets for eventual use in peptide-based immunotherapy.

Prickett et al. identified five immunodominant T cell epitopes in Ara h 2 by generating multiple CD4+ T cell lines from peripheral blood mononuclear cells (PBMCs) of an HLA-diverse peanut-allergic population and utilizing proliferation and ELISPOT assays to determine peptide recognition patterns. Recognition of candidate peptides in peanut-allergic individuals was confirmed by measuring production of T_H2-type cytokines, including IL-4 and IL-5, in response to peptide exposure. Additionally, candidate peptides were screened for

IgE binding when exposed to peanut-allergic sera, and there was no IgE binding detected, further supporting the concept that peptide-based immunotherapy minimizes IgE cross-linking and the risk for side effects [37]. In another study performed by the same group, several T cell epitopes in Ara h 1 were also identified by generating CD4+ T cell lines using PBMCs of 18 peanut-allergic subjects and utilizing different proliferation assays. Ten core epitopes within Ara h 1 were selected and mapped, and seven short peptide sequences (< 20 amino acids in length) encompassing all identified core epitopes were designed. Basophil activation in response to these peptides was measured as an indirect assessment of IgE cross-linking. None of the designed peptide sequences induced basophil activation at any concentration tested [38].

Ramesh et al. identified candidate T cell epitopes of Ara h 1 by utilizing novel methods of in silico MHC class II binding prediction and in vitro HLA-binding assays performed in parallel. Thirty-six candidate peptides were identified and underwent stimulation assays, which led to T cell proliferation in all peptides with peanut-allergic subjects responding to a greater number of peptides compared to nonallergic controls. Furthermore, cytokine levels (including IL-4, IL-13, and IFN-γ) were measured in tissues exposed to each peptide, and all peptides stimulated a T_H2-dominant response. To demonstrate safety, a mix of seven peptides associated with the strongest T cell responses was used in basophil degranulation assays utilizing whole blood from peanut-allergic subjects. There was no evidence of degranulation at any of the three tested concentrations, suggesting that these peptides had no IgE cross-linking capabilities [39].

To demonstrate proof of concept in a murine model, one study treated peanut-sensitized mice with Ara h 1 peptides. The mice were subsequently challenged with peanut administered intraperitoneally to induce anaphylaxis. Different clinical parameters, including body temperature changes and vascular leakage, were measured during anaphylaxis, and samples of skin, spleen, lymph nodes, bone marrow, and peritoneal lavage were obtained after the mice were sacrificed. The treatment group demonstrated significantly decreased anaphylaxis measurements with peanut challenge, and Ara h 1-specific T cell levels were reduced in treatment group tissue samples compared to controls [40]. In a separate study performed by the same group, an Ara h 1 peptide containing one T cell epitope recognized by C57BL/6 mice was identified. This peptide was administered in six different doses, ranging from 0.01 to 300 μg, to groups of sensitized mice. Anaphylaxis was induced with peanut challenge and mice in the placebo group demonstrated higher anaphylaxis scores compared to peptide-treated mice. Mice treated with 100 μg of Ara h 1 peptide experienced the highest level of protection. Results indicated that immunotherapy with just one peptide could protect against anaphylaxis in a dose-dependent manner [41].

Recombinant Allergen Vaccines

Background

The use of recombinant native allergens has also been considered for use in immunotherapy for food allergy. Similar to peptide-based immunotherapy, in order to minimize the risk for adverse reactions, the best designed recombinant food allergens have a decreased or eliminated ability to bind IgE while retaining the ability to stimulate T cell responses that is comparable to native proteins. The use of recombinant allergens in immunotherapy has the potential to induce desensitization with shorter courses of treatment compared to the use of whole allergens, as higher doses can be administered with little or no dose escalation required. The production process involves denaturation of the recombinant wild-type allergen, production of recombinant allergen fragments, or formation of mosaics through reassembly of allergen fragments that leads to reduced IgE binding and decreased allergenic potential through disruption of conformational IgE epitopes. Recombinant mutants can also be produced by removal of amino acids or peptides involved in IgE binding [42]. Meanwhile, allergen T cell epitopes are preserved, which allows for IgG antibody production and promotion of regulatory and T_H1 immunomodulatory effects. Clinical trials examining the safety and efficacy of using recombinant allergens in immunotherapy in the treatment of environmental allergies including Birch and Timothy grass pollens have already been published with encouraging results [43, 44].

Peanut Allergy

Ara h 1, Ara h 2, and Ara h 3 are three major peanut allergens whose immunodominant T cell epitopes have been mapped out using synthetic peptides and sera from a large cohort of peanut-allergic individuals. Additionally, the amino acid sequences needed for IgE binding by these epitopes have been identified, allowing for the production of recombinant hypoallergenic variants of Ara h 1, Ara h 2, and Ara h 3 in *Escherichia coli*. In vitro studies have shown that modified peanut allergens exhibit decreased IgE binding compared to wild-type allergens while still retaining the ability to stimulate T cell proliferation [45, 46].

Bacterial adjuvants are potent stimulators of the T_H1 immune response and can be co-administered with hypoallergenic peanut proteins as an adjuvant to improve their efficacy. The efficacy and safety of this technique has been explored in several studies. The effects of three times weekly subcutaneous administration of three modified peanut allergens (mAra h 1, mAra h 2, and mAra h 3) with heat-killed *Listeria monocytogenes* (HKLM) used as an adjuvant was investigated by Li, et al. in a murine model of peanut allergy. C3H/HeJ mice given the combination of modified allergens plus HKLM

over a 4-week period not only had reduced plasma peanut-specific IgE levels, but when undergoing an intragastric peanut challenge, they had significantly reduced changes in core body temperature, serum histamine levels, and measurements of bronchial constriction compared to sham-treated mice, reflecting a decreased incidence and severity of anaphylaxis. The association between changes in cytokine profiles and the protective effects of treatment was also assessed. A statistically significant reduction in IL-4, IL-5, and IL-13 as well as an increase in IFN-γ in splenocytes of mice treated with mAra h-13 plus HKLM was noted. This trend was not identified in groups treated with HKLM or mAra h 1–3 alone [47].

Another study by Li et al. utilized heat-killed *Escherichia coli* that produced engineered Ara h 1, 2, and 3 proteins (HKE-MP123). This mixture was administered rectally to mice and, following an intragastric peanut challenge, mice treated with HKE-MP123 were found to have significantly reduced plasma histamine levels and anaphylactic symptoms compared to sham-treated mice. This protective effect lasted up to 10 weeks after treatment was discontinued [48].

Given these encouraging results, a suspension (EMP-123) comprised of three recombinant peanut allergens (Ara h 1, 2, and 3) encapsulated within inactivated *E. coli* was developed for human use. A Phase I non-randomized, open-label trial was performed to assess safety and efficacy. EMP-123 was given rectally in weekly dose escalations over 10 weeks in ten peanut-allergic adults followed by three biweekly maximal doses. Of the ten patients, five patients (50%) experienced adverse reactions severe enough to prevent them from completing the trial, including two anaphylactic events. The other five patients experienced mild or no symptoms. Assessing immunologic differences between the two patient groups revealed that median baseline peanut-specific IgE and Ara h2-specific IgE levels were significantly higher in those individuals who were unable to complete the trial. But due to the frequency and severity of adverse reactions, further drug development has been halted and the trial authors concluded that additional modifications to the allergens and/or dosing regimen would be needed to improve safety [49].

Egg Allergy

As previously discussed, the major allergens identified in hen's eggs include ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), lysozyme (Gal d 4), and albumin (Gal d 5) [23]. Ovomucoid is a heat-stable glycoprotein made of 186 amino acids arranged into three tandem domains, and recombinant varieties have been developed utilizing site-directed mutagenesis. In one study, five genetically modified third domain mutants received single or double amino acid substitutions within IgE and IgG binding epitopes. Western blot and ELISA assays showed decreased IgE and IgG binding and reduced allergenicity compared to native, unaltered allergens [50]. In an in vivo study, native ovomucoid third domain antigen (DIII) was used to sensitize Balb/c mice. Desensitization was then performed using intraperitoneal injections of modified ovomucoid third domain antigen administered over a 3-week time period. Mice were then challenged to ovomucoid DIII antigen to provoke anaphylaxis. Desensitized mice experienced elimination of all anaphylaxis symptoms, lower plasma histamine levels, and increased T_H1-type cytokine levels compared to controls, suggesting that modified ovomucoid third domain antigen could be used to attenuate the allergic response in DIII-sensitized mice [51].

Because there is some evidence to suggest that protein glycation influences susceptibility to food allergies, another study utilized glycated ovalbumin administered over a 4-week time period to ovalbumin sensitized BALB/c mice. The carbohydrate moieties tested included mannose, glucomannan, and galactomannan which were compared to non-glycated ovalbumin and phosphate-buffered saline as controls. Following an ovalbumin oral challenge to induce anaphylaxis, mannose- and glucomannan-treated mice were found to have significantly lower anaphylaxis symptom scores and specific IgE levels and increased levels of T-reg cells. The study authors concluded that the use of mannose and galactomannan-glycated ovalbumin could potentially be used in treatment of egg allergy [52].

Fish Allergy

For the development of immunotherapy for fish allergy, the FAST project (Food Allergy Specific Immunotherapy) focused on the carp allergen, parvalbumin (Cyp c 1), which harbors extensive IgE cross-reactivity with paravalbumins from multiple other fish species [53]. Cyp c 1 is a protein that exhibits extraordinary stability despite exposure to heat and digestion and has been identified as a major allergen in fish. A hypoallergenic form of Cyp c 1 was developed by utilizing Ca^{2+}-binding site mutagenesis, which resulted in loss of secondary structure and an average 1000-fold reduction in IgE binding capability when tested with sera from fish-allergic patients but still retained its immunogenicity [54].

A first-in-human Phase I/IIa double-blind, randomized, placebo-controlled trial (NCT02017626) was performed looking at the use of mCyp c 1 in fish-allergic subjects. A low side effect profile and positive immunological response were noted, although the data has not yet been formally published [55]. But, as an extension to this study, a Phase IIb multinational clinical trial (NCT02382718) was developed where 45 fish-allergic subjects were randomized to receive either treatment with subcutaneous injections of mCyp c 1 or placebo. The buildup phase consisted of ten injections administered over an 8-week period until a maintenance dose of 60 μg was reached, which was then continued for 4 months. The primary endpoint was efficacy as determined by a change

in the amount of fish needed to induce a clinically significant allergic reaction. The study was completed in April 2017 with formal publication still pending.

DNA-Based Vaccines

Background

Another distinct approach to treatment of food allergy is to focus on allergen exposure in the form of DNA rather than proteins. Prior studies looking at vaccine injections of a plasmid DNA (pDNA) vector showed that humoral and cellular responses could be induced to the encoded antigen. The pDNA sequence is taken up by antigen-presenting cells (APCs) which transcribe and translate the antigen-specific DNA into protein which is presented on the cell surface to T cells via MHC complexes [56, 57]. Since DNA vaccination preferentially induces a T_H1 immune response, its use in allergic disease is being investigated, since a weighted imbalance towards a T_H2 immune response has been thought to be a major causative factor in the development of atopic disease [58].

Peanut Allergy

In a murine model, oral gene delivery using Ara h 2 pDNA complexed with chitosan, which is a nonimmunogenic polysaccharide that improves gene adhesion and transport in the gut, led to gene expression in intestinal epithelium. Immunized peanut-sensitive AKR mice showed a significant reduction in allergic symptoms including symptom measurements of anaphylaxis, food-specific IgE levels, and serum histamine levels following challenge with Ara h 2 protein compared to controls. The authors concluded that chitosan-pDNA nanoparticles could be a potential oral treatment option for food allergies [59]. In another study by Li et al., different mouse strains were administered intramuscular injections of plasmid DNA encoding Ara h 2. Following 3 weeks of immunization, injections of Ara h 2 elicited anaphylactic reactions in C3H/HeSn mice while immunized ARK/J and BALB/c mice remained asymptomatic. These studies suggest that the type of immune response to pDNA immunization is strain-dependent, which brings about concerns that significant interindividual variations in efficacy using pDNA in the treatment of food allergy may exist [60]. Additionally, results from human trials using DNA-based vaccines have suggested somewhat disappointing immunomodulatory effects, and finding a better delivery system to enhance the potency of DNA vaccines is needed [61].

In an attempt to address the issue of poor efficacy and potency, one study investigated the use of intradermal injections of pAra h 2 or p Ara h 2 pretreated with poly-L-lysine (PLL) in BALB/c mice either before or after sensitization with Ara h 2 protein. Mice pretreated with pAra h 2 or PLL-pAra h 2 all had lower levels of Ara h 2-specific IgG1, IgG2a, and IgE after being sensitized to Ara h 2 protein, however, mice in the PLL-pAra h 2 group had significantly lower antibodies compared to the pAra h 2 group. Mice that received injections after protein sensitization also had significantly decreased levels of Ara h 2-specific antibodies, with the PLL-pAra h 2 mice showing a greater effect. There were also increased numbers of CD207+ dendritic cells, Tregs, IFN-γ, and IL-10 levels in mice given PLL-pAra h 2 compared to pAra h 2. The authors concluded that modification of pAra h 2 with PLL resulted in improved immunomodulatory effects compared to using naked pDNA alone and may represent a possible strategy to enhance efficacy in future clinical applications [62].

In another attempt to augment the efficacy of DNA vaccines, lysosomal-associated membrane protein-1 (LAMP-1) has been included in DNA plasmids to elicit enhanced immunomodulatory effects via greater production of antibodies and cytokines. In one study ARA h1,2,3-LAMP Vax, a multivalent peanut (Ara h1, h2, h3) LAMP DNA plasmid vaccine was administered intradermally for 4 weeks to peanut-sensitized C3H/HeJ mice. A peanut challenge was performed 3 weeks after therapy to induce anaphylaxis. Resulting data showed that ARA h1,2,3-LAMP Vax treated mice exhibited 70% lower peanut-specific IgE levels compared to controls. Treated mice also had lower anaphylaxis symptom scores, higher core body temperatures, and lower plasma histamine levels following peanut challenge compared to controls. The splenocytes from treated mice produced more IFN-γ and IL-10. This suggests that ARA h1,2,3-LAMP Vax offers a protective effect against allergic reactions in peanut-sensitized mice and may have therapeutic potential in the treatment of peanut allergy [63]. An ongoing phase 1 randomized, placebo-controlled trial is currently underway to assess the safety and efficacy of ARA h1,2,3-LAMP Vax in peanut-allergic adults (NCT02851277).

Conclusion

Food allergy remains a growing public health concern worldwide, with the incidence of food allergy increasing over time. There is currently no FDA-approved, accessible, safe, and effective treatment for food allergy, and standard of care is strict dietary avoidance and use of epinephrine in emergency situations. Although food immunotherapy via the oral, sublingual, and epicutaneous routes are under active clinical investigation, standardized protocols are still lacking on how such therapies should be implemented into daily practice, and the risk of IgE-mediated reactions remains a substantial concern. The use of peptide-based immunotherapy, recombinant allergen vaccines, or DNA-based vaccines have demonstrated promise as a way to improve safety and enhance efficacy of immunotherapy but further studies, particularly in human subjects, will be necessary to confirm safety and efficacy.

Compliance with Ethical Standards

Conflict of Interest Dr. Kulis reports grants from NIH and from DoD, outside the submitted work. Dr. Yang declares no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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