



Vaccines as therapies for food allergies

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Abstract

Food allergy is a growing public health epidemic with few available treatments beyond allergen avoidance and rescue medications for accidental exposures. A major focus of therapeutic development for food allergies is allergen-specific immunotherapy (AIT) in which patients are exposed to increasing amounts of allergen in controlled dosing to induce desensitization or tolerance. The work of the past few decades has culminated in the recent FDA approval of a peanut product for oral AIT for peanut allergies. Despite these advances, current AIT protocols are cumbersome, take a long time to reach clinical benefit and often have significant side effects. Therefore, there is a great need to develop new therapeutics for food allergy. One area of research aims to improve AIT through the use of adjuvants which are substances traditionally added to vaccines to stimulate or direct a specific immune response. Adjuvants that induce Th1-polarized and regulatory immune responses while suppressing Th2 immunity have shown the most promise in animal models. The addition of adjuvants to AIT may reduce the amount and frequency of allergen required to achieve clinical benefit and may induce more

long-lasting immune responses. In this chapter, we highlight examples of adjuvanted AIT and vaccines in development to treat food allergies.

Abbreviations

AIT	allergen-specific immunotherapy
alum	aluminum adjuvant
EPIT	epicutaneous immunotherapy
LAMP	lysosomal-associated membrane protein
MPLA	monophosphoryl lipid A
OIT	oral immunotherapy
PLGA	poly-D-L-lactide-co-glycolide
SCIT	subcutaneous immunotherapy
SLIT	sublingual immunotherapy
TLR	toll-like receptor
Treg	regulatory T cell
TSLP	thymic stromal lymphopoietin
VLP	virus-like particle



1. Introduction

Food allergy prevalence has increased significantly over the past 2 decades and is estimated to affect 8% of children and 4–10% of adults in the United States (Gupta et al., 2019). Food allergy can have a large impact on quality of life for both patients and their families due to dietary limitations and significant social and emotional impact. Until recently, the only approved treatments for food allergy included allergen avoidance and rescue medications, including epinephrine and antihistamines, for emergency use following accidental exposure (Sicherer & Sampson, 2014). There have been major advances in the development of therapeutics for food allergy in recent years, culminating in 2020 with the approval of the peanut immunotherapy product Palforzia, the first FDA-approved therapeutic specifically for food allergy.

This chapter will focus on IgE-mediated food allergy which accounts for the majority of allergic reactions to foods. In the case of food allergies, sensitization results from the development of Th2-polarized cellular immune responses to food and the generation of allergen-specific IgE antibodies. The immunological development of these responses occurs when pro-inflammatory cytokines termed alarmins (IL-25, IL-33 and thymic stromal lymphopoietin (TSLP)) are released by epithelial cells, which results

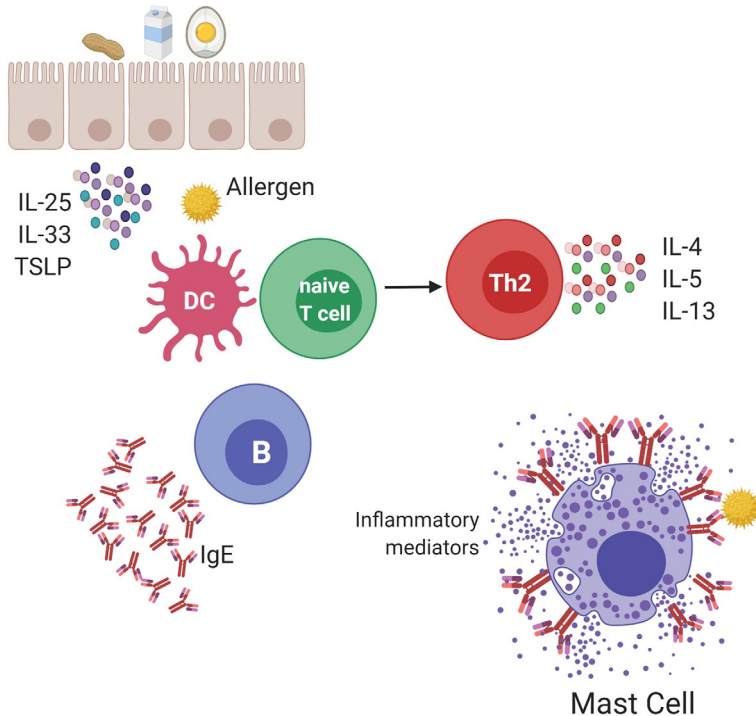


Fig. 1 Mechanisms of allergic sensitization. Sensitization to foods occurs when food allergens cross epithelial borders and the secretion of innate cytokines, including IL-25, IL-33 and TSLP lead to the generation of Th2 cells and IgE-producing B cells. Allergic reactions occur when allergen binds to IgE that is loaded on the FcεR1 on mast cells. Crosslinking of IgE leads to rapid mast cell degranulation and release of inflammatory mediators. Figure created using BioRender.com.

in dendritic cells (DCs) that promote the differentiation of naïve $CD4^{+}$ T cells into Th2 cells. Th2 cells produce key cytokines (IL-4, IL-5, IL-9, and IL-13) that are responsible for mediating allergic reactions and inducing B cell class switching to IgE (Fig. 1). Allergen-specific IgE binds to its receptor (FcεR1) on mast cells and basophils. When these cells are exposed to allergen, IgE antibodies on the cell surface cross-link, leading to degranulation and the release of inflammatory mediators, such as histamine, prostaglandins and leukotrienes which cause the clinical manifestations of allergic reactions and anaphylaxis (Tordesillas, Berin, & Sampson, 2017).

A number of biologics are currently being tested for the treatment of food allergies. Omalizumab (Genentech), an anti-IgE antibody currently FDA approved for allergic asthma, is under clinical investigation for food

allergy. Monoclonal antibodies that target the cytokines that mediate allergic disease are also in clinical trials for food allergy, including the anti-IL-4 receptor alpha antibody dupilumab (Regeneron Pharmaceuticals) and the anti-IL-33 antibody etokimab (AnaptysBio) (Chinthrajah et al., 2019; Rial, Barroso, & Sastre, 2019). While these biologics suppress allergic disease in a non-allergen-specific manner, the majority of the therapeutics in clinical development are allergen specific, and this chapter will focus on these allergen-specific immunotherapies and vaccines for food allergy.



2. Allergen-specific immunotherapy

Allergen-specific immunotherapy (AIT) has demonstrated great promise for the treatment of allergic disease. In simple terms, AIT involves the exposure of an allergic individual to gradually increasing doses of allergen with the goal of inducing desensitization or tolerance (Wood, 2016). The first reported use of AIT was in 1911 for the treatment of grass pollen allergies (Noon, 1911). Since these first attempts over 100 years ago, numerous advances have been made including the development of AIT protocols for many allergens and the investigation of multiple routes of delivery. AIT has been well-studied for treatment of allergic rhinitis, conjunctivitis, asthma and venom allergy, and in some cases it has been demonstrated to induce tolerance (Larenas Linnemann, 2012). The development of AIT protocols for food allergy has increased in the past decade, with the recent approval of an oral immunotherapy (OIT) product and protocol for peanut allergy (Kim & Burks, 2020; Palisade Group of Clinical Investigators et al., 2018; Sampath, Sindher, Alvarez Pinzon, & Nadeau, 2020). Unlike AIT for allergic rhinitis, there is a lack of evidence that OIT can induce long-term tolerance to food allergy in a majority of cases. However, remaining on maintenance therapy does provide significant benefits to patients. It is likely that further research of patients who remain on food AIT for longer times may uncover protocols capable of inducing long-term effects, as has been reported for AIT for allergic rhinitis (Sampath et al., 2020).

Traditionally AIT has been based on either subcutaneous (SCIT) or sublingual (SLIT) administrations of allergen extracts. Allergen can also be delivered orally (OIT) or epicutaneously (EPIT), and these routes have been most extensively studied for food allergies. In SCIT, allergen is injected at increasing doses each week for several weeks, followed by monthly maintenance injections that continue for at least 3 years to achieve clinical protection. SCIT has demonstrated positive clinical effects, especially in

treating seasonal allergic rhinitis, and is widely used in the clinic today for aeroallergens (Jutel, Kosowska, & Smolinska, 2016). However, while early clinical trials of SCIT with unmodified food extracts suggested similar efficacy, they also resulted in adverse events, including anaphylaxis (Nelson, Lahr, Rule, Bock, & Leung, 1997). Current research on SCIT for food allergy is focusing on modifying food allergens to generate hypoallergenic proteins that can be used in AIT without triggering reactivity (Feuille & Nowak-Wegrzyn, 2018; Jongejan, van Ree, & Poulsen, 2016). Because SCIT for food AIT is not currently considered safe, the majority of the clinical development of food AIT has adapted SCIT protocols to deliver allergen via sublingual, oral and epicutaneous routes which have improved safety profiles.

Safety and efficacy of food AIT is variable, depending on the route of administration and the type and dose of food used. Two studies directly compared OIT and SLIT and demonstrated increased efficacy with OIT (Keet et al., 2012; Narisety et al., 2015). Recent efforts have been focused on developing OIT and EPIT products protocols for food allergy, but these are mostly still considered investigational. The United States Food and Drug Administration recently approved a standardized peanut product for OIT (Palforzia, Aimmune Therapeutics) in peanut allergic children 4–17 years of age based on extensive clinical studies (Palisade Group of Clinical Investigators et al., 2018). Sixty-seven percent of participants in the Phase III clinical trial passed an oral food challenge of at least 600 mg peanut protein, compared to only 4% in the placebo arm. Adverse events were common during peanut OIT, with 1 in 13 participants experiencing an adverse event requiring epinephrine usage and 1 in 15 participants withdrawing from therapy (Grzeskowiak, Tao, Knight, Cohen-Woods, & Chataway, 2020). Adverse events mostly occurred during the up dosing portion of therapy, and epinephrine use was low during the long-term maintenance phase. In a Phase III clinical trial of EPIT using the Viaskin Peanut patch in children aged 4–11 years, significantly reduced systemic side effects were observed compared with peanut OIT with no incidents of severe anaphylaxis (Fleischer et al., 2019). However, efficacy was more modest, with 35.3% of participants receiving EPIT passing a food challenge, compared with 13.6% in the placebo arm. Both of these AIT protocols require long durations of treatment to provide a protective benefit, and to date no food AIT approaches have demonstrated the induction of tolerance in a large percentage of patients. Clinical trials have investigated the length of protection or sustained unresponsiveness, which is determined by passing an oral food

challenge after stopping AIT for a period of weeks to months. These studies have demonstrated that as the period of allergen avoidance after AIT increases, the rate of sustained unresponsiveness decreases (Vickery et al., 2014). Protocols for OIT for other foods are being developed, including ongoing clinical trials and in clinical practice without FDA approval (Freeland et al., 2017; Wasserman et al., 2019). Further study is needed to refine protocols for food AIT and determine markers to indicate continued desensitization or induction of tolerance.

The immune mechanisms by which food AIT induce desensitization or tolerance have not been fully defined; however, many correlates of protection have been determined in both animal and human studies. AIT is associated with increased allergen-specific IgG4 and IgA, which may indicate a role for blocking antibodies in protection (Jones et al., 2017; Keet et al., 2012; Narisety et al., 2015). The protective effects often correlate with changes to allergen-specific T cells. AIT results in reduced production of allergen-specific Th2 cytokines (IL-4, IL-5, IL-13), which may be mediated by specific deletion of allergen-specific Th2 cells or induction of anergy due to constant allergen exposure (Wambre et al., 2014). AIT-induced regulatory T cells (Tregs) can also suppress allergic immune responses through production of cytokines, including IL-10 and TGF- β , that can also mediate inhibition of Th2 immunity and suppression of mast cell reactivity (Feuille & Nowak-Węgrzyn, 2018). There may also be a role for regulatory B cells in suppression of Th2 cell function and modulation of the humoral immune response (Kim et al., 2016; van de Veen et al., 2016). AIT could also suppress allergic reactivity through the induction of allergen-specific Th1 immunity, as IFN- γ suppresses Th2 cells and reduces IgE production (Wambre et al., 2014). Successful AIT has been associated with high levels of IFN- γ and IL-10, suggesting the induction of antigen-specific Th1 and regulatory cells as key components mediating the suppression of allergic disease (Akdis & Akdis, 2014). These immunologic changes are often transient with AIT and require continuation on maintenance therapy to retain the immunological and clinical benefits (Gorelik et al., 2015).

While food AIT has shown significant promise for the future of treating food allergies, at present, there has not been a protocol that has demonstrated the induction of tolerance or cure in a significant number of patients. Responder rates for OIT, SLIT and EPIT vary greatly, and these therapies also carry risks of adverse events. OIT has shown more instances of allergic reactions than EPIT which has been demonstrated to be well-tolerated (Dupont et al., 2010; Feuille & Nowak-Węgrzyn, 2018). However, despite

Table 1 Immunotherapies and vaccines under development for food allergy.
Selected references

<i>Allergen-specific immunotherapy (AIT)</i>	
Subcutaneous (SCIT)	Nelson et al. (1997)
Sublingual (SLIT)	Keet et al. (2012); Narisety et al. (2015)
Oral (OIT)	Freeland et al. (2017); Palisade Group of Clinical Investigators et al. (2018)
Epicutaneous (EPIT)	Dupont et al. (2010); Fleischer et al. (2019); Jones et al. (2017)
<i>Modified hypoallergenic allergens</i>	
Allergoids	Carnes, Gallego, Moya, and Iraola (2018); van der Kleij et al. (2019)
Peptides	Dhar and Larché (2019); Prickett et al. (2011); Rupa and Mine (2012); Simms et al. (2015)
Recombinant allergens	Bindselev-Jensen et al. (2017); Wood et al. (2013); Zuidmeer-Jongejan et al. (2015)
<i>Genetic vaccines</i>	
mRNA vaccines	Roesler et al. (2009); Scheiblhofer, Thalhamer, and Weiss (2018)
DNA vaccines	Li et al. (1999); Li, Saade, and Petrovsky (2012)
Targeted DNA vaccines	
• LAMP1 fusion	Li, Song, Su, Heiland, and Sampson (2015); Liu (2011)
• DEC-205 fusion	Maaske et al. (2016)
<i>Microbial products</i>	
Probiotics	Barletta et al. (2013); Meijerink et al. (2012)
Heat-killed bacteria	Li, Srivastava, Grishin, et al. (2003); Li, Srivastava, Huleatt, et al. (2003); Wood et al. (2013)
<i>TLR ligand-based adjuvants</i>	
CpG	Kulis, Gorentla, Burks, and Zhong (2013); Srivastava et al. (2016); Zhu, Kandimalla, Yu, and Agrawal (2007)

Continued

Table 1 Immunotherapies and vaccines under development for food allergy.—cont'd
Selected references

MPLA/GLA	Graver et al. (2016) ; Soos et al. (2016)
<i>Particle delivery systems</i>	
Virus-like particles	Storni et al. (2020)
Liposomes	Bednar et al. (2017) ; Orgel et al. (2017)
<i>Biodegradable polymers</i>	
• PLGA	Jilek, Walter, Merkle, and Cortes (2004)
• Chitosan	Li et al. (2009) ; Roy, Mao, Huang, and Leong (1999)
Nanoemulsions	O'Konek et al. (2018, 2020)

the risk of adverse events, the benefit conferred by AIT is sufficient for many patients ([Anagnostou et al., 2014](#); [Feuille & Nowak-Węgrzyn, 2018](#)). Despite the major advances in recent years, there remains an unmet need to develop other therapies for food allergy, which may increase efficacy and longevity of protection with reduced side effects. There are multiple modalities currently under development that aim to improve food AIT by reducing the amount and/or frequency of allergen exposure and increasing long-term desensitization or tolerance induction. A wide variety of therapeutics to enhance AIT for food are under development, including use of modified food allergens, adjuvants and vaccines. A selection of these therapies are listed in [Table 1](#).



3. Modified hypoallergenic proteins for the treatment of food allergy

The safety of food AIT may be increased through the development of hypoallergenic modified food allergen proteins that can be used for AIT without inducing reactivity ([Yang & Kulis, 2019](#)). Various methods have been used to modify allergen structure to reduce IgE binding to prevent allergenicity. Modified allergens include heated or baked food, recombinant proteins engineered for reduced IgE binding, and peptides. Modified allergens are also under investigation for use in vaccines to treat food allergy, which will be discussed in more detail later in this chapter.

High heat can denature conformational epitopes of proteins, which may lead to decreased capacity to bind to IgE. This is also supported by milk and egg allergic individuals who can tolerate these foods in baked form, as evidence suggests that regular consumption of baked egg or milk can promote the development of tolerance in egg and milk allergic patients (Lemon-Mule et al., 2008). Similarly hydrolyzed egg protein has been tested in OIT in both mice and humans, and demonstrated reduced allergenicity (Giavi et al., 2016; Hacini-Rachinel et al., 2014). This suggests that for some allergens, the safety of OIT could be improved through the use of extensively heated allergen, leading to desensitization to the native form of the allergen.

3.1 Allergoids

Allergens can also be modified to reduce allergenicity through crosslinking and polymerization of denatured native allergens to form allergoids (Carnes et al., 2018). Allergoids retain the linear epitopes required for T cell activation but lack conformational epitopes required for IgE binding. Allergoids have been mostly used in AIT for aeroallergens (Huser et al., 2017). The application of allergoids for food AIT remains to be determined, but preclinical studies have generated allergoids to reduce the allergenicity of many different food allergens such as egg, milk, Brazil nut and peanut (Bencharitiwong, van der Kleij, Koppelman, & Nowak-Wegrzyn, 2015; Salgado, Casadevall, Puignero, & Queral, 1996; Smit et al., 2013; van der Kleij et al., 2019; Villas-Boas, Fernandes, de Lima Zollner, & Netto, 2012). These studies provide proof of concept for the use of these hypoallergenic allergen proteins in vaccines for food AIT.

3.2 Peptide-based immunotherapy

Another method to reduce the risk of adverse reactions to food AIT is utilizing peptides (approximately 10–20 amino acids in length) comprising T cell epitopes of allergens that cannot cross-link IgE on mast cells or basophils. The goal of this approach is to identify peptides that contain epitopes to activate T cell responses without binding to IgE. AIT with these peptides has been shown to induce the positive outcomes of AIT without causing anaphylaxis (Gupta, Kumar, Das, & Dwivedi, 2014).

Peptides are chosen by epitope mapping in which potential T cell epitopes are identified by cloning allergen-specific T cells from large cohorts of allergic patients. These T cell lines are used to screen a library of synthetic peptides spanning the length of the allergen's proteins. Dominant T cell

epitopes are identified based on the capacity of the peptides to stimulate T cells (Ali & Larche, 2005). Immunotherapy with T cell epitopes has been used to treat cat, house dust mite and bee venom allergies and has demonstrated the ability to confer altered T cell responses to whole allergen extracts (Hoyne, O'Hehir, Wraith, Thomas, & Lamb, 1993; Muller et al., 1998; Oldfield, Larche, & Kay, 2002).

Peptide-based vaccines are in an earlier stage of development for food AIT. T cell epitopes have also been identified for food allergens, including egg, peanut and cow's milk (Prickett et al., 2011; Ruiter et al., 2006; Yang & Mine, 2009). Studies in murine models of allergy to egg and peanut have demonstrated the potential for safety and efficacy of synthetic peptide-based vaccines for food allergy (Rupa & Mine, 2012; Simms et al., 2015). A Phase I clinical trial of PVX108, an intradermal vaccine containing T cell epitopes of major peanut allergens Ara h1 and Ara h2, is ongoing in Australia (ACTRN12617000692336), and preliminary results indicate that it is safe and tolerable in individuals with peanut allergy (Dhar & Larché, 2019).

3.3 Recombinant allergen vaccines

Similar to peptide-based immunotherapy, recombinant food allergens have been engineered to have decreased ability to bind IgE but still retain the epitopes necessary to stimulate T cell responses (Bannon et al., 2001; King et al., 2005; Mine, Sasaki, & Zhang, 2003). Recombinant allergens have been tested in both traditional food AIT and vaccines as alternatives to native allergens (Li, Srivastava, Grishin, et al., 2003; Li, Srivastava, Huleatt, et al., 2003; Rupa & Mine, 2006a). Because of their reduced binding to IgE, higher doses of allergen can be administered without reactivity (Zhernov, Curin, Khaitov, Karaulov, & Valenta, 2019). This may eliminate the need for long periods of dose escalation, thus reducing the time of treatment required to induce desensitization.

Generation of recombinant proteins with reduced capacity to bind IgE can be accomplished by several methods, including removal of amino acids in the IgE-binding epitopes, denaturation to alter protein folding, or reassembly of allergens to form mosaics (Valenta, Linhart, Swoboda, & Niederberger, 2011). Clinical trials for the treatment of aeroallergens including birch and grass pollens using recombinant allergens have demonstrated safety and efficacy, and this approach is currently being investigated for the treatment of food allergies (Jutel et al., 2005; Pauli et al., 2008).

For peanut allergy, IgE-binding domains and immunodominant T cell epitopes have been mapped for the major allergens Ara h1, Ara h2 and Ara h3. Identification of these epitopes allowed for the design of recombinant variants of these allergens which do not contain IgE-binding motifs but retain their T cell stimulatory function (Bannon et al., 2001). A Phase I trial was completed for EMP-123, a vaccine composed of three recombinant peanut allergens (Ara h1, Ara h2, and Ara h3) encapsulated in heat and phenol inactivated *Escherichia coli* (Wood et al., 2013). Adverse events were common in peanut allergic subjects, with 20% having severe allergic reactions. Another peanut allergy vaccine, HAL-MPE1, a chemically modified peanut extract adsorbed on aluminum hydroxide and delivered by the subcutaneous route, was well-tolerated in a small group of 17 peanut allergic adults in Denmark (Bindslev-Jensen et al., 2017). A larger Phase I study of HAL-MPE1 is currently ongoing in the United States and Canada (NCT02991885). Modified hypoallergenic egg proteins have been developed and have been shown to induce desensitization without reactivity in mouse models (Rupa & Mine, 2006b). A European Union funded Food Allergy Specific Immunotherapy (FAST) project focused on the development of SCIT for food allergy by modifying allergens to improve safety and tolerability. Fish and peach allergy were chosen, as these are each predominantly caused by a single defined allergen, Cyp c 1 and Pru p 3, respectively (Zuidmeer-Jongejan et al., 2012), and modified recombinant fish protein has been tested in a subcutaneous vaccine for patients with fish allergy (Zuidmeer-Jongejan et al., 2015). Both Phase I (NCT02017626) and Phase IIb (NCT02382718) studies have been completed, but results have not been published (Jongejan et al., 2016).



4. mRNA and DNA vaccines

Another novel approach to the treatment of food allergies is the use of mRNA or DNA vaccines. In contrast to vaccines that contain protein antigens, genetic vaccines contain only mRNA or DNA (in the form of plasmids) which are taken up by cells in the patient and translated into protein by the host machinery. The protein is then loaded into MHC complexes on the cell surface to present to T cells (Tang, DeVit, & Johnston, 1992). Plasmid DNA or mRNA vaccines induce immune responses similar to those associated with viral infections, including the induction of strong Th1-polarized immune responses (Roesler et al., 2009). This approach is in development for many types of vaccines and has been tested in numerous

clinical trials mainly for anti-cancer vaccines (Weide, Garbe, Rammensee, & Pascolo, 2008). Because these vaccines induce strong Th1-biased immunity, mRNA and DNA vaccines have been pursued as therapeutic allergy vaccines, and their ability to modulate established Th2-polarized allergic immunity has been investigated (Scheibelhofer et al., 2018).

The first studies of DNA vaccines for allergy involved intramuscular immunization with plasmid DNA encoding house dust mite allergens driven by a CMV promoter. This vaccine was shown to prevent sensitization to house dust mite and later demonstrated to suppress established allergic disease (Weiss et al., 2006). This genetic vaccine approach has also been tested in multiple murine models of food allergy. In a study by Li et al., three weekly immunizations with plasmid DNA encoding Ara h2 induced complete protection against challenge with Ara h2 in certain strains of mice (Li et al., 1999). A major caveat of this approach is that results from human clinical trials using DNA vaccines have generally not demonstrated impressive efficacy, and better routes of vaccine delivery may be required to enhance efficacy of DNA vaccines (Li et al., 2012).

Multiple strategies have been used to improve DNA and mRNA vaccines. The addition of LIMP (lysosome membrane protein) or LAMP (lysosomal-associated membrane protein) sequences in the DNA plasmid targets the allergen to the endolysosomal compartment and results in increased loading of the allergen peptides onto MHC-II molecules. A next-generation DNA vaccine platform encodes LAMP-1 in plasmids with allergens to elicit enhanced efficacy of DNA vaccines (Liu, 2011). Upon immunization, antigen presenting cells take up the vector and translate the allergen that is associated with LAMP-1. In contrast to traditional DNA vaccines which primarily result in presentation on MHC-I and corresponding activation of CD8⁺ T cell responses, the LAMP-1-allergen complexes induce significantly more CD4⁺ T cell responses and increase production of antibodies and cytokines. LAMP-1 DNA vaccines for Japanese Red Cedar allergy were shown to induce Th1-polarized immune responses in mice, and a Phase I clinical trial demonstrated safety in humans (Su, Connolly, Marketon, & Heiland, 2016; Su et al., 2017). This approach is also under development for the treatment of peanut allergy. ARA-LAMP-Vax, a DNA plasmid vaccine encoding multivalent peanut allergen (including Ara h1, h2 and h3) and LAMP DNA, reduced peanut-specific IgE and clinical symptoms of anaphylaxis following peanut challenge in mice (Li et al., 2015). The peanut-specific cellular immune responses were

also shifted from Th2 cytokines to IFN- γ and IL-10, suggesting that protection was associated with immune modulation. The safety and tolerability of this multivalent peanut-LAMP-1 DNA vaccine (ASP0892; Astellas Pharma Inc.) is currently being evaluated in a Phase I clinical trial for adults with peanut allergy ([NCT02851277](#)). Utilization of the LAMP-Vax platform should prevent adverse reactions to the vaccine, as the synthesized allergen-LAMP fusion protein is directed to the lysosome without secretion of native allergen from the cell.

In addition to targeting the endolysosomal compartments, DNA vaccines have been engineered to express proteins that will target the vaccine directly to DCs ([Chen et al., 2016](#)). DNA vaccines encoding DEC-205 allergen fusion proteins have been demonstrated to suppress allergic asthma in mice ([Maaske et al., 2016](#)). This approach resulted in increased Tregs, compared with non-targeted vaccines, and reactivity to the vaccine was also reduced. These studies provide evidence that targeted allergy vaccines may increase safety by sequestering allergen away from effector cells to avoid reactivity to the vaccine.

Plasmid DNA-based vaccines have been tested in a number of clinical trials without demonstrating serious adverse events. However, there are concerns regarding safety of this approach to vaccination, namely that persistence of the plasmid DNA or integration into the genome could lead to adverse immunological effects due to long-term expression of the antigen/allergen ([Weiss et al., 2006](#)). Methods to improve safety include the use of DNA vaccines encoding hypoallergenic allergen fragments, mutant allergens, or DNA encoding CD4⁺ or CD8⁺ T cell epitopes that do not bind IgE ([Weiss et al., 2006](#)). mRNA vaccines may offer an improved safety profile as these are degraded by host RNases and cannot integrate into the host genome.

Genetic vaccines have the potential to enhance both the safety and efficacy of food AIT. The exposure of allergic patients to allergen DNA or mRNA rather than allergen protein may reduce the adverse reactions that occur with traditional food AIT. No native allergen is secreted, so exposure of effector cells to allergen may be more limited. Additionally, this vaccine-based approach would eliminate the requirement for daily allergen exposure and may reduce the duration required to achieve clinical benefit. Because of the evidence that genetic vaccines for allergens induce Th1-polarized immune responses as well as regulatory T cells, this approach has the potential to provide long-term protection.



5. Adjuvants

Adjuvanted immunotherapy is an emerging area of research to improve AIT. The most common disadvantages of current AIT for food allergy are dosing frequency, amount of allergen required, adverse side effects, and the lack of long-term protective effects once AIT is discontinued. All of these issues could potentially be ameliorated by augmenting food AIT with immunological adjuvants. Adjuvants are substances added to vaccines to enhance or direct the immune response, typically through the activation of innate signaling pathways (McKee & Marrack, 2017). Traditional vaccines that contain inactivated pathogens or protein subunits often include adjuvants to enhance the magnitude as well as to direct the skewing of the humoral and cellular immune responses to antigen.

Adjuvants could improve AIT in multiple ways. Adjuvants may decrease the amount of allergen required to see a clinical benefit, thus reducing side effects due to allergen exposure. Adjuvants elicit more durable immunological responses, so the intervals between doses could be increased from daily to monthly or longer. Because vaccine adjuvants induce durable immunity lasting for years, this approach has the potential to dramatically increase the length of suppression of reactivity. With the development of novel next-generation vaccine adjuvants that activate distinct immunological pathways, there is the opportunity for rational drug design to develop adjuvants that specifically suppress the Th2 immune responses associated with allergic disease.

Aluminum adjuvant (alum) has been the most commonly used adjuvant in vaccines for infectious agents for almost 100 years (Glenny, Pope, Waddington, & Wallace, 1926). Alum has also been included in SCIT for aeroallergens and bee venom where repeated administrations ultimately result in the generation of blocking IgG antibodies and suppression of Th2 immunity and allergic reactivity (Jensen-Jarolim, 2015). Alum is likely not a good candidate for food AIT, as SCIT has been demonstrated to be unsafe for food AIT. Alum also can result in an initial elevation of IgE antibodies and Th2 immune responses which could enhance reactivity. Therefore, there is ongoing research aimed at finding the best adjuvants to augment AIT for foods.

Adjuvants often stimulate immunity through the activation of innate immune pathways that are critical for host defense (McKee & Marrack, 2017). Many adjuvants were discovered and continue to be engineered

based on the understanding of how the innate immune system recognizes pathogen-associated molecular patterns (PAMPs). The innate immune system is comprised of many different pattern recognition receptors that recognize these PAMPs to be able to quickly mount an immune response to invading pathogens. Through the activation of different innate receptors, including toll-like receptors (TLRs) and Nod-like receptors (NLRs), adjuvants can induce varied immune responses. Current research suggests that the optimal adjuvants for AIT would stimulate Th1 immune responses and/or suppress Th2 immunity.

5.1 Microbial flora and probiotics

The microbiome plays critical roles in the regulation of homeostasis and is important for many aspects of human health. Microbial flora have been implicated in the regulation of immune responses to food antigens, as microbes that colonize the skin and gut mucosa interact with and influence the immune system. Reduced microbial exposure, as investigated with the use of antibiotics or germ-free mice, can result in increased Th2 immune responses and a greater severity of allergic reactions in murine models (Stefka et al., 2014). Further studies have demonstrated that a reduced ability of the host to respond to microbial stimulation also can enhance allergic disease, as mice lacking toll-like receptor 4 (TLR4), which recognizes bacterial cell wall components, have increased severity of allergic reactions (Bashir, Louie, Shi, & Nagler-Anderson, 2004). Investigation of the role of the microbiome in food allergy has also uncovered differences in the composition of the gut microbiome of food allergic and non-allergic patients, as well as an association of factors that influence microbiome content (antibiotic use, breastfeeding, route of birth, etc.) with the incidence of allergic disease (Stephen-Victor, Crestani, & Chatila, 2020). Lacking proper balance of bacterial in the gut microbiome or having a reduced ability to interact with microbial products may be critical for the development or maintenance of allergic sensitization. Certain bacteria have been associated with protection from the development of food allergy and animal studies suggest that treating mice with specific bacteria can reduce allergic responses (Iweala & Nagler, 2019). Taken together, this suggests that interactions between the immune system and the gut microbiome are important in food allergy and beneficial microbes or microbial-derived substances may be useful adjuvants for food AIT.

Ingestion of probiotics may have immunomodulatory effects and may be useful adjuvants to improve the efficacy of food AIT. Probiotics have

been used safely for years as nutritional supplements. However, it is still not clear how probiotics modulate immunological and metabolic processes, so the use of probiotics may also carry risks (Doron & Snyderman, 2015). *Lactobacilli* and *Bifidobacteria* are commonly used in probiotic formulations and have demonstrated an ability to lessen the severity of peanut allergy reactions in mice (Barletta et al., 2013; Meijerink et al., 2012). Suppression of allergic reactivity by supplementation with these bacteria was associated with increased regulatory T cells, suppression of Th2 cytokines and reduction in allergen-specific IgE. This suggests that in conjunction with AIT, microbial products may be useful for modulation of allergic immunity.

5.2 Heat-killed bacteria

Heat-killed bacteria retain the adjuvant properties described above, however these bacterial products may be safer, as innate immune responses can be activated without the risk of exposure to live bacteria. This approach has been tested for the treatment of peanut allergy. Subcutaneous immunization with peanut proteins (Ara h1, 2, and 3) and heat-killed *Listeria monocytogenes* reduced peanut-specific IgE and Th2 cytokines and protected from oral allergen challenge in mouse and dog models of peanut allergy (Frick, Teuber, Buchanan, Morigasaki, & Umetsu, 2005; Li, Srivastava, Huleatt, et al., 2003). A similar study utilized heat-killed *E. coli* engineered to express Ara h1, 2, and 3 proteins and demonstrated similar effects at suppressing allergic disease (Li, Srivastava, Grishin, et al., 2003). This led to a Phase I clinical trial of EMP-123, recombinant peanut allergens (Ara h 1, 2, and 3) encapsulated within heat-killed *E. coli*. Further modifications of this approach are required, as significant adverse events occurred in half of the patients (Wood et al., 2013).

5.3 TLR ligand-based adjuvants

The use of TLR ligands, as opposed to whole pathogens, may induce more specific effects and be less inflammatory. Microbial cellular components or metabolic products are also known to play a role in immune modulation (Belkaid & Hand, 2014). These specific components have the potential to increase safety, as they do not require the administration of living microbes. The adjuvant properties of these products are dependent upon stimulation of these innate receptors and activation of downstream signaling pathways to initiate immune responses (Georg & Sander, 2019). As many of these microbial products stimulate Th1 type immunity, they have

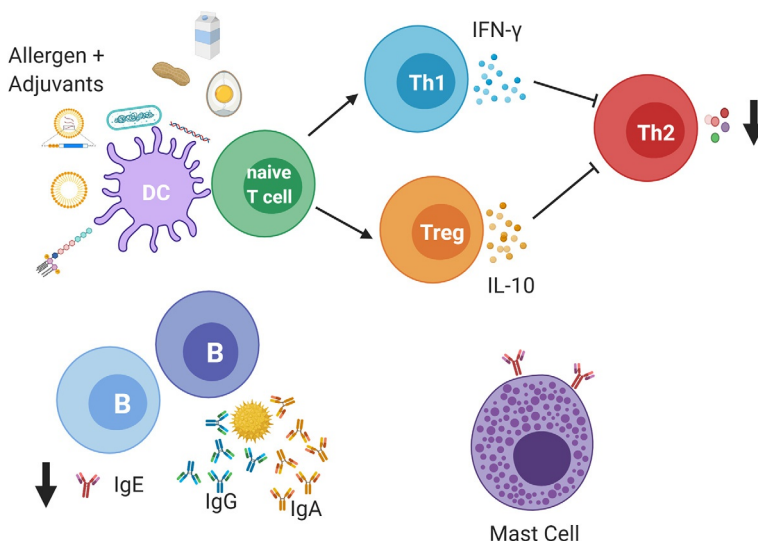


Fig. 2 Mechanisms of adjuvanted immunotherapy. The addition of adjuvants to AIT or allergy vaccines stimulates innate immune receptors on DCs to lead to generation of allergen-specific Th1 or Treg cells as well as the suppression of allergen-specific Th2 cells. Allergy vaccines can also decrease allergen-specific IgE, leading to reduced IgE on mast cells to prevent degranulation and release of inflammatory mediators. Increased IgG and IgA function as blocking antibodies to prevent allergen from initiating the allergic cascade. Figure created using BioRender.com.

been tested for their ability to suppress allergic disease, and preclinical models have suggested that using Th1-polarizing TLR ligands may be effective at suppressing established Th2 immunity (Fig. 2).

Unmethylated CpG oligodeoxynucleotides are TLR9 ligands which promote strong Th1-polarized immune responses in both mice and humans and have been studied for use as adjuvants in AIT with promising results (Chu, Targoni, Krieg, Lehmann, & Harding, 1997; Simons, Shikishima, Van Nest, Eiden, & HayGlass, 2004). CpG also has been shown to suppress Th2 responses such as IL-5, IL 13, IgG1 and IgE and has been formulated into nanoparticles and virus-like particles (VLP) to enhance delivery. Subcutaneous administration of CpG VLPs formulated with house dust mite extract were tested in Phase I and II clinical trials (Klimek et al., 2011; Senti et al., 2009). Patients treated with CpG-adjuvanted VLPs had significantly reduced symptoms of allergic rhinitis and asthma, and conjunctival provocation tests demonstrating a 10-fold increase in amount of allergen that could be tolerated. CpG has also demonstrated promise in

preclinical work in murine models of peanut allergy where it both prevented sensitization and suppressed established allergic disease (Kulis et al., 2013; Srivastava et al., 2016; Zhu et al., 2007). Therapeutic efficacy correlated with suppression of Th2 immune responses and IgE as well as increased IFN- γ and Tregs (Fig. 2). Redirection of the immune response with Treg induction may suggest long-term functionality of this approach. The ability of this Th1-polarizing adjuvant to reduce Th2 immune responses and provide clinical benefit in patients with allergic disease suggests that other adjuvants that induce Th1 immune response are also worth further investigation for the treatment of food allergy. The TLR4 agonist monophosphoryl lipid A (MPLA) is a derivative of lipopolysaccharide (LPS) from the cell wall of Gram-negative bacteria (Qureshi, Takayama, & Ribí, 1982). Animal model studies using MPLA or a synthetic analog glucopyranosyl lipid A (GLA) as a vaccine adjuvant indicate that it induces Th1-polarized immune responses (de la Torre, Baeza, Najera, & Zubeldia, 2018), and MPLA/GLA-adjuvanted vaccines have been effective in controlling pollen allergies in humans and peanut allergies in mice (Graver et al., 2016; Soos et al., 2016). The use of adjuvants that increase Th1, Tregs and IL-10 or antagonize Th2 immune responses are being considered as potential adjuvants for AIT for allergic asthma or rhinitis and may also be useful in augmenting AIT for foods.



6. Nanoparticle vaccine delivery systems and adjuvants

In recent decades nanoparticle technologies have been investigated in immunomodulatory therapies to enhance efficacy and reduce toxicity mainly in the context of treatments for infectious diseases and cancer. Many nanoparticles (defined as being 1–1000 nm in diameter) have been designed as vaccine delivery systems, but some formulations have also been shown to have adjuvant activity to enhance or direct the cellular immune responses to the antigen. Nanoparticles can be formulated from many different components, including biodegradable polymers such as poly-D-L-lactide-co-glycolide (PLGA), chitosan, oils and surfactants (Jilek et al., 2004; Smith, Simon, & Baker, 2013). Nanoparticles such as virus-like particles and the oil-in-water emulsion MF59 are currently in use, while many more are under development (Del Giudice, Rappuoli, & Didierlaurent, 2018; Storni et al., 2020). The physicochemical properties of nanoparticles (size, charge, hydrophobicity, stability) vary based on components and formulation. Engineering nanoparticles to change these properties alters the mucosal delivery of allergen

as well as the magnitude and the skewing of the immune response (Wong et al., 2015). Therefore, these physiochemical properties are key components of rationale design of the next-generation adjuvants capable of enhancing AIT.

Nanoparticles have been formulated with additional vaccine adjuvants to increase the magnitude of the induced immune response as well as promote skewing toward Th1. CpG containing virus-like particles were studied in Phase I and II clinical trials for patients with house dust mite allergy. This therapy improved symptoms of allergic rhinitis and asthma and required significantly less allergen to be administered to see this effect (Klimek et al., 2011; Senti et al., 2009). These studies demonstrate that the addition of adjuvants in AIT can improve efficacy while reducing the dose of allergen required for achieving clinical benefit.

Nanoparticles may also enhance oral immunization when engineered to be resistant to degradation by stomach acid (De et al., 2014). Studies have demonstrated that encapsulation of allergens or allergen extracts within nanoparticles induces IL-10 responses and promotes tolerance (Srivastava et al., 2016). The addition of Th1-polarizing adjuvants to nanoparticle formulations may improve the immunomodulatory function of allergen-containing nanoparticles, as was demonstrated in a murine model of peanut allergy in which CpG and peanut containing nanoparticles reduced allergic reactivity to peanut (Srivastava et al., 2016). Nanoparticles made of the polysaccharide chitosan have been used to improve oral delivery of allergen to suppress food allergy in mice (Li et al., 2009; Roy et al., 1999). Nanoparticles can also be engineered to target specific cell types, including liposomes formulated to express a ligand for CD22, a Siglec expressed on B cells. The combination of allergen and CD22 targets the liposomes to allergen-specific B cells to induce apoptosis, resulting in loss of allergen-specific IgE and induction of tolerance in mouse models of peanut allergy (Bednar et al., 2017; Orgel et al., 2017).

Nanoscale emulsion adjuvants are oil-in-water emulsions formulated with different oils, solvents and surfactants. The squalene-based nano-emulsion MF59 has been used clinically in seasonal and pandemic influenza vaccines. MF59 is a potent adjuvant and induces Th1-polarized cell-mediated immune responses (Seubert et al., 2011). The soybean oil based nanoemulsion W805EC has been shown to safely induce potent Th1- and Th17-polarized mucosal, humoral and cellular immune responses after its intranasal administration in both animal models and humans (Makidon et al., 2008; Stanberry et al., 2012). The adjuvant activity of this nano-emulsion formulation is primarily dependent on the positive charge of

the nanoscale droplets that facilitates binding to negatively mucin proteins in the nasal mucosa and penetration of the mucous layer. The droplet size facilitates cellular uptake and trafficking to the draining lymph nodes, resulting in induction of innate and adaptive immune responses (Farazuddin et al., 2019; Makidon et al., 2012). Intranasal immunotherapy with a nanoemulsion adjuvant and peanut or milk proteins suppressed reactivity at allergen doses that did not have any effect when delivered without adjuvant (O'Konek et al., 2018, 2020). Nanoemulsion adjuvants are antigen-sparing and induced protection in mice after only three administrations of 20 µg of allergen which is significantly lower than milligram quantities required for OIT. Additionally, this approach achieved sustained unresponsiveness for over 4 months following the last dose. Taken together, the data from these vaccine approaches indicate that the use of adjuvants may increase the efficacy and duration of protection of AIT while reducing adverse reactions from allergen exposure.

Nanoparticles modulate immune responses through multiple diverse mechanisms. Nanoparticles function as delivery systems that can be targeted to lymphoid organs based on particle size dependent tissue trafficking and enhanced antigen uptake by antigen presenting cells. Macrophages and DCs take up the particles and associated allergen allowing for a swift and strong immune response while keeping the allergen shielded so it does not bind to IgE on mast cells triggering an allergic reaction (Scholl et al., 2004). Nanoparticles often promote the stability and persistence of antigens, and this depot effect results in gradual and sustained release of antigen. The immunomodulatory effects associated with nanoparticles typically are associated with activation of the innate immune system through pattern recognition receptors including TLRs, resulting in the release of cytokines, chemokines and other mediators that regulate immune responses (Liu, Hardie, Zhang, & Rotello, 2017). In addition to immune activation and enhancement, nanoparticles can also have immunosuppressive effects on certain immune cells, including B and T cells or antigen presenting cells (Smarr et al., 2016). Nanoparticle-induced secretion of TGF-β and IL-10 induce regulatory cells, including Tregs, which can further dampen the immune response. While nanoparticles have shown promise in mouse models of food allergy, this approach has not yet been well-studied in humans, and investigation of both efficacy and safety of this approach to adjuvanted AIT should be a priority.

While several types of vaccine adjuvants have been evaluated for their ability to modulate allergic immune responses, there is the potential for

many other adjuvants and vaccines to be beneficial for treating food allergy. Adjuvants that downregulate immune responses that promote allergy, such as Th2 cytokines and IgE, or enhance regulatory and Th1-polarized responses are currently in preclinical development and may be useful in novel adjunctive therapies to improve AIT for food allergies.



7. Conclusion

Food allergy remains a growing public health concern worldwide, with the incidence of food allergy increasing over time and few approved treatment options. There is currently only one FDA-approved treatment for peanut allergy, and standard of care is strict dietary avoidance with the use of epinephrine following accidental exposures. Although multiple food AITs 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. The use of vaccine adjuvants may improve AIT by reducing the amount of allergen and number of doses required to achieve protective effects, but further studies, particularly in human subjects, will be necessary to confirm safety and efficacy. Food allergy vaccines represent a hope for a future in which immunology-driven precision medicine can improve therapy for food allergies.

Conflict of interest

J.J.O. is an inventor on patent application that has been submitted for a nanoemulsion adjuvant for the suppression of allergic disease that is described in this chapter (PCT/US2015/054943). This technology has been licensed to Blue Willow Biologics, and the University of Michigan has a financial interest in Blue Willow Biologics. J.J.L. has no conflicts of interest to disclose.

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