

Potential therapies for peanut allergy

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Objective: To review the investigated therapies for peanut allergy beyond avoidance measures and self-injectable epinephrine.

Data Sources: A PubMed search was performed using the Keywords *peanut allergy* and *therapy*. Additional citations were generated by surveying the reference lists of the pulled articles.

Study Selection: More than 120 articles were reviewed and references were selected based on their relevance to the subject matter.

Results: Peanut allergy affects more than 1% of the US population and is increasing in prevalence. During the past 15 years multiple therapies have been researched and many have provided promising results. Sustained oral tolerance over desensitization is the goal, and most therapies are unable to demonstrate this because they are currently in their relative infancy. Therapeutic options should be safe, easily administered, and relatively inexpensive. To minimize risk, many therapies will require investigation of combined modalities.

Conclusions: Peanut allergy is a challenging diagnosis for physicians because few treatment options are available. However, it seems plausible that new offerings may become accepted therapy within the next decade. The ability of a patient to tolerate amounts of peanut in an unintentional ingestion without experiencing anaphylaxis would offer peace of mind to patients and families living with peanut allergy.

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INTRODUCTION

Food allergy is of increasing concern in westernized countries because its incidence appears to be increasing. More specifically, peanut allergy among US children was found to have doubled in prevalence, from 0.4% in 1997 to 0.8% in 2002, and an estimated 1.1% of the US population, or 3 million people, are allergic to peanut, tree nuts, or both.¹ Peanut's ubiquitous presence in the diet of most industrialized coun-

tries makes it difficult to avoid. The annual incidence of unintentional ingestion among schoolchildren in Montreal, Quebec, Canada, was reported to be 14.3%.² In addition, peanut allergy tends to be both more severe in its degree of reaction and less likely to be outgrown than other food allergies. Of 63 reported food anaphylaxis deaths in the United States from 1994 to 2006, peanut was the trigger in 59% of the fatalities.^{3,4} Although peanut allergy is estimated to resolve for approximately 20% of children by school age,^{5,6} it recurs in 8%, with significantly higher rates among individuals who continue to avoid peanuts after allergy resolution.⁷

Certain populations, such as very young children and those who frequently do not prepare their own meals, may be at higher risk of unintentional ingestion. Elevated risk-taking behaviors among adolescents and college students place them at substantially increased risk for food-related anaphylaxis. A 2009 survey of 287 University of Michigan students with a probable or known food allergy revealed that only 40% of

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students with a clinical history of food-related anaphylaxis maintained self-injectable epinephrine and less than 10% reported always carrying it with them.⁸ Furthermore, 60% of individuals who reported having a reaction continued to knowingly ingest their identified food allergens. A similar survey of the same age group, with 75% of respondents reporting peanut allergy, found that nearly 50% ate foods they were allergic to because the food looked good and they wanted to eat it.⁹

Despite our increased understanding of peanut and other food allergies during the last several decades, the treatment has remained constant—education on strict avoidance measures, including intense scrutiny of food labels, wearing of medical alert devices, and the use of self-injectable epinephrine. Constant vigilance against unintentional ingestions significantly affects the lives of patients and their families, with a poorer quality of life compared with children with rheumatic diseases and insulin-dependent diabetes mellitus.^{10,11} With that in mind, some physicians have postulated that the controlled risks of more novel therapies performed in an allergist's office or hospital setting outweigh the uncontrollable risks of unintentional ingestion with resultant anaphylaxis in the outside environment.^{12,13}

It is against that backdrop of controllable and uncontrollable risk that we review the investigated therapies for peanut allergy beyond avoidance measures and self-injectable epinephrine. A PubMed search was performed using the Keywords *peanut allergy* and *therapy*. Additional citations were generated by surveying the reference lists of the pulled articles. More than 120 articles were reviewed and references were selected based on their relevance to the subject matter.

PEANUT ALLERGY AND THE INDUCTION OF TOLERANCE

Peanut allergy occurs in 2 phases: sensitization and allergic reaction. As peanut proteins pass through the gastrointestinal tract, they are transferred to dendritic cells (antigen-presenting cells) and presented as peptide fragments by class II major histocompatibility complex molecules, which bind to T-cell receptors on naive T_H cells. In those people predisposed to allergic sensitization, this process leads to the synthesis of peanut specific IgE antibodies through release of cytokines (interleukin 4 [IL-4], IL-5, and IL-13) by T_H2 cells and B-cell stimulation. These peanut specific IgE antibodies then bind to the high-affinity surface IgE receptors (FcεRI) of mast cells and basophils. On subsequent ingestion, allergic reaction occurs when the specific IgE antibodies on these cells are cross-linked by peanut protein, signaling cellular degranulation, and release of histamine, platelet-activating factor, prostaglandins, and leukotrienes.¹⁴

Most therapies covered on the following pages act, at least in part, to reverse T_H2 predominance toward a more prominent T_H1 profile. Ultimately, tolerance, defined as specific suppression of cellular or humoral immune responses to an antigen through oral antigen administration, would be preferred to antigen desensitization, which would require con-

tinued, potentially even life-long, dosing without permanent changes in the immune response. Oral tolerance occurs through 2 separate mechanisms. Low-dose antigen induces tolerance through regulatory T cells and down-regulatory cytokines, such as IL-4, IL-10, and transforming growth factor β. High-dose tolerance occurs in lymphocytes via anergy (absence of costimulatory signals between CD28 receptors and CD80/CD86 receptors on antigen-presenting cells) or clonal deletion through Fas-mediated apoptosis, which can be blocked by IL-12.¹⁵ In patients who outgrew their peanut allergy and therefore acquired tolerance, cytokine levels more closely resembled the T_H1 profiles (interferon γ [IFN-γ] and tumor necrosis factor α [TNF-α]) of individuals without history of peanut allergy than those with active peanut allergy and a T_H2 profile (IL-4, IL-5, and IL-13).¹⁶

THERAPIES

The idea of treating food allergy beyond mere avoidance is not a new concept. In 1908, Schofield¹⁷ reported successfully treating a boy with severe egg allergy through the gradual increase in egg concentration in daily pills. The procedure took 8 months, beginning with 1/10,000th of an egg and ending with the boy eating a whole egg every day.

Nearly 80 years later, the first peanut desensitization was described, albeit unpublished, in 4 peanut allergic patients who successfully tolerated daily peanut ingestion after a gradual buildup phase.¹⁸ Separately, rush immunotherapy with subcutaneous injections of peanut extract resulted in a marked reduction in symptom scores after double-blind, placebo-controlled peanut challenge in 3 patients who had completed a challenge before and after 4 weeks of immunotherapy. The 3 patients also had an average 33,000-fold decrease in titrated skin test sensitivity, whereas the placebo treatment group was observed to increase 36-fold. There was a 13.3% incidence of systemic reactions reported during the 4-week rush immunotherapy period.¹⁹ However, in a follow-up study using the previous rush protocol followed by 12 months of maintenance immunotherapy, systemic reactions during the maintenance phase occurred as frequently (39%) as the rush protocol (23%).²⁰ Furthermore, the increased tolerance to peanut achieved after rush immunotherapy was partially or completely lost in half of the treated patients because of necessary maintenance dose reductions after systemic reactions. Although demonstrating some potential for allergy reduction, the risks involved with subcutaneous immunotherapy to peanut were deemed unacceptable and the pursuit of alternative therapies for peanut allergy largely occurred as a result.

Oral Immunotherapy

Peanut oral immunotherapy (OIT) has been one of the most pursued research modalities because of successes in egg, milk, and fish OIT.²¹ Many studies and case reports exist detailing successful tolerance to peanut, with doses ranging from 300 mg/d to 40 g 3 times per week.^{13,22–25} In a 2009

open-label study, Jones et al²⁵ reported on 29 peanut allergic children ages 1 to 16 years who underwent escalation and buildup to 300 mg or 1,800 mg based on results of oral food challenges (OFCs) followed by maintenance peanut OIT. After an average of 4.7 months (range, 4–22 months) of maintenance therapy, 93% (27/29) reached a total peanut dose of 3.9 g on OFC with only mild symptoms compared with only 26% tolerating 50 mg on the initial day of escalation. Peanut specific IgE levels were significantly decreased by the end of the observation period, and IgG4 levels were significantly elevated. Titrated skin prick tests and basophil activation studies were both significantly decreased at 6 months. Secretion of IL-10, IL-5, IFN- γ , and TNF- α from peripheral blood mononuclear cells increased during 6 to 12 months. Interestingly, T-cell microarrays performed before and 6 months after starting OIT showed downregulation of apoptotic gene pathways similar to previous observations in allergen immunotherapy, suggesting a possible mechanism of OIT. The OFC results after 3 years of maintenance therapy are forthcoming, and double-blind, placebo-controlled studies are under way. The demonstration of long-term tolerance vs desensitization remains to be seen, but immunomodulation appears to be taking place with reduction in T_H2 cytokines and increased T-regulatory cell function reported in the most recent analysis.²⁶

In an analysis of the safety of this OIT protocol, Hofmann et al²⁷ reported that 93% of patients experienced adverse symptoms during the escalation day, 46% during the buildup period, and 3.5% during home dosing. Upper respiratory tract symptoms (eg, sneezing, nasal itching, or congestion; rhinorrhea; and mild laryngeal symptoms) were most likely to be encountered among all stages, with 79%, 29%, and 1.2% reported, respectively. Treatment for home doses with diphenhydramine, albuterol, or both was required in 0.7%, with associated epinephrine use in only 0.02% (2/10,184) of home doses. Subsequent analysis of 7 patients reporting reactions during home dosing led to recommendations to (1) avoid dosing for less than 3 days during concurrent illness, (2) closely monitor and control asthma and allergic rhinitis with appropriate medications if needed, (3) take the daily dose with food, (4) limit exertion for 2 hours after dosing, and (5) closely monitor during menses, particularly in the presence of infection or exercise.²⁸ Table 1 provides a summary of peanut oral immunotherapy studies.

Peanut sublingual immunotherapy (SLIT) is currently in the earliest stages of investigation, and, like OIT, it also builds on previous success with other food allergens. A 2005 randomized double-blind, placebo-controlled study of hazelnut SLIT found that patients had significant increases in their sensitivity thresholds to hazelnut allergen via double-blind, placebo-controlled food challenge after 3 months of maintenance immunotherapy (approximately 13 mg/d), and this therapy was well tolerated.²⁹ In an ongoing, open-label peanut SLIT clinical trial at Duke University, 4.7% of the 2,554 doses given during the escalation phase were associated with mild reactions (eg, transient oropharyngeal itching, isolated

hives, pruritus, or sneezing).³⁰ No significant changes were found in peanut specific IgE, IgG, IgG4, or skin prick test wheal diameter compared with the placebo treatment group at 4 months into buildup dosing,³¹ consistent with findings in aeroallergen SLIT. Primary efficacy through OFCs at 12 months and beyond is yet to be reported.

Both OIT and SLIT appear promising in regard to offering a long-lasting solution to peanut allergy. However, as the authors of these studies have often mentioned, many questions regarding these therapies remain unanswered, and given the intensity of the protocols and the risks, particularly during the escalation phase, adaptation to the clinical setting should be avoided until further insights are available.

Anti-IgE Therapy

Nonspecific immunomodulation in peanut allergy was previously studied using TNX-901, a humanized mouse IgG1 monoclonal anti-IgE antibody that binds to the CH₃ domain with high affinity and inhibits binding to the Fc ϵ RI on mast cells and basophils.³² In a randomized, double-blind, placebo-controlled, dose-ranging study, 84 patients with a history of peanut allergy underwent a double-blind, placebo-controlled peanut challenge and were then assigned to receive 4 doses of either 150, 300, or 450 mg of TNX-901 or a placebo subcutaneously every 4 weeks. After the series of injections, the mean threshold dose of peanut on final OFC increased from baseline in a dose-dependent manner, although only the 450-mg group reached statistical significance when compared with the placebo group ($P < .001$). Participants in the 450-mg group initially tolerated an average of 178 mg of peanut flour (approximately half a peanut) but subsequently tolerated 2,805 mg (almost 9 peanuts) after therapy. Furthermore, 21% in the 300-mg group and 24% in the 450-mg group were able to tolerate at least 8 g of peanut flour (approximately 24 peanuts). However, the therapeutic response was inconsistent, and 25% were unable to tolerate a dose of 0.5 g of peanut flour, despite receiving the highest dose of TNX-901. These results were not associated with peanut specific or total serum IgE concentrations. In a separate double-blind, phase 2 trial of omalizumab (Xolair; Genentech, San Francisco, California), 9 omalizumab and 5 placebo patients underwent a double-blind, placebo-controlled peanut challenge before and after 22 weeks of injections every 2 to 4 weeks.³³ The study was discontinued after safety concerns in OFC participants, prohibiting definitive conclusions. However, the limited data appeared to show an overall trend toward tolerability in the omalizumab group over placebo. Separate phase 2 clinical trials are currently under way.

Anti-IgE therapy for food allergy would likely require injections indefinitely and as a result would be resource intensive and costly. It may serve a better role as a supplemental therapy, particularly during escalation phases of immunotherapy, to minimize the risk of anaphylaxis related to the peanut dosing. Phase 1/2 studies with peanut OIT are planned for the near future.

Table 1. Summary of Peanut Oral Immunotherapy Studies^a

Study	No. of patients	Age, y	Starting dose	Time to maintenance	Success rate	Adverse events	Comments
Mansfield ¹³	1	6	250 μ g	4 whole kernels (8 g) 3 times daily for 8 weeks	1/1	1/1, W, S	Therapy decreased to 4 whole kernels twice daily, peanut specific IgE decreased from >100 IU to 42 IU at 12 months
Clark et al ²²	4	9–13	5 mg	800 mg peanut protein for 10–20 weeks, approximate doubling of dose every 2 weeks	4/4	3/4, OI, N, AP, RC, V, S	No epinephrine required during OIT, all participants tolerated 800 mg of protein per day for 6 weeks with tolerance of >10 peanuts during postintervention challenges (16- to 160-fold increase from baseline)
Patriarca et al ²³	1	38	5.6 mg on day 1	40 g peanuts per day for 7 days in hospital, then 40 g peanuts 3 times per week	1/1	0/1 (Daily loratadine and ranitidine during rush and 2 weeks into maintenance phase)	Peanut SPT result became negative at 6 months, no significant changes in peanut specific IgE or IgG4
Jones et al ²⁵ and Hofmann et al ²⁷	29	1–16	100 μ g	300 mg peanut protein, dose increased by 25 mg every 2 weeks from 50 mg after initial 1-day escalation. Dose increased to 1800 mg if peanut IgE was >2 kU/L after 12 months on maintenance dose	27/29 (Total peanut dose of 3.9 g on OFC. 2 patients tolerated 2.1 g)	26/28, escalation, RC, N, AP, S, L, V, W, 46% risk during dose buildup. RC, S, AP, N, W 3.5% risk/dose maintenance, RC, S, AP, N, W	39 Patients completed initial day escalation, 6 withdrew for personal reasons, 4 due to allergic reactions, no DBPCFC performed to confirm peanut allergy prior to protocol, significantly decreased peanut IgE, increased IgG4, decreased SPT response at 6 months, increased levels of IL-5, IL-10, IFN- γ , and TNF- α
Blumchen et al ²⁴	23	3–14	0.4–24 mg (1/100th of the eliciting DBPCFC dose)	500 mg crushed peanut for 0–20 months (median, 7 months) after rush protocol in hospital for up to 7 days	14/22 (4 treatment failures, 3 dropouts, 1 partial responder)	25/317, doses rush phase, W, V, S, RC, L, 160/6137 doses long-term buildup with maintenance, W, V, S, RC, L (only objective symptoms reported)	Final DBPCFC performed after 2 weeks of peanut avoidance with approximately 4-fold increase from baseline, high-risk participants with 2/3 (two thirds) having asthma, slow buildup appeared safer than rush protocol, only children with lower peanut specific IgE were successful for the rush protocol, reduced peanut SPT diameter after OIT, increased IgG4 levels, decreased IL-4, IL-5, and IL-2 production

Abbreviations: AP, abdominal pain; DBPCFC, double-blind, placebo-controlled food challenge; IFN- γ , interferon γ ; IL, interleukin; L, laryngeal; N, nausea; OFC, oral food challenge; OI, oral itching; OIT, oral immunotherapy; RC, rhinoconjunctivitis; S, skin; SPT, skin prick test; TNF- α , tumor necrosis factor α ; V, vomiting; W, wheezing.

^a Approximately 240 mg of peanut protein equals 1 whole peanut.

Probiotics

Multiple studies have been performed evaluating the effects of probiotics on atopic dermatitis, but studies in food allergy have been limited. The main sources of probiotics come from dairy products containing *Lactobacillus* and *Bifidobacterium* species, and, in theory, probiotics help to maintain a proper intestinal microbial balance. In some cases, inactivated probiotics may be equally efficacious compared with live forms and, therefore, preferable to activated forms because of a theoretically lower risk of infections. In Brown Norway rats sensitized to peanut, *Lactobacillus casei* given 1 week before sensitization and daily for the 6-week study period caused peanut specific IgE, IgG1, and IgG2a to increase, leading the authors to conclude that there was no protective effect of probiotics in the prevention of allergic response to peanut in this particular animal model.³⁴ Flinterman et al³⁵ studied the effects of a mixture of *Lactobacillus* and *Bifidobacterium* species vs placebo in a therapeutic trial of 13 infants and toddlers who were sensitized to at least 2 common food allergens. In vitro, the probiotics elevated T_H1 cytokines and regulatory IL-10, whereas, after 3 months of administration, the ex vivo effects caused a decreased production of IL-10, TNF- α , and IL-6. Peanut specific IgE was unchanged as was skin prick test wheal size.

Intriguingly, in 2008, Zhang et al³⁶ discovered significant benefits in peanut allergic mice after treatment with a koji mold (found in miso, soy sauce, and sake) probiotic derived from fermenting soybeans with *Aspergillus oryzae* and lactic acid bacteria. Mice were treated for 4 weeks to the activated and irradiated sterilized forms of the probiotic followed by peanut challenge in conjunction with sham treatment and naive mice groups. The treated mice showed significant reduction in incidence and degree of anaphylaxis, as well as plasma histamine levels, after challenge compared with the sham treatment group. Peanut specific IgE levels were also significantly lower, whereas T_H2 cytokines were diminished and IFN- γ was increased when compared with the sham treatment mice. Furthermore, acute toxicity studies found the product to be safe after histologic analysis and 14 days of observation in rats who ingested the equivalent of 100 times the dose used in humans. The inactivated form was of comparable effect to the activated form, thus minimizing the potential for infection in children.

Soy-Based Immunotherapy

Soybean storage proteins share similar amino acid structure with peanut proteins. The use of homologous soybean protein for immunotherapy in peanut-sensitized mice was compared with peanut immunotherapy containing Ara h 1 and Ara h 2.³⁷ No significant difference was found in symptom scores after challenge between the 2 groups, and no significant differences were found in IgG2 and total IgE levels. Both groups had notable elevations in IFN- γ and TNF- α levels compared with the sham treatment group, signaling a shift toward a T_H1 response. One limitation in this study was that the mice were not sensitized to peanut orally but rather intraperitoneally,

and the results may not correlate to sensitization in humans. Also, this therapy would likely be limited in those individuals with concomitant soy allergy.

Chinese Medicine

Although multiple approaches within traditional Chinese medicine have been used to treat asthma, no traditional Chinese therapy for food allergy existed before the development of a new mixture labeled Food Allergy Herbal Formula 1 (FAHF-1).³⁸ This formula contained extracts of 11 herbs selected largely for their anti-inflammatory properties and their roles in treatment of symptoms related to food-induced anaphylaxis, such as colic or vomiting. Mice sensitized to peanut were treated with FAHF-1 and showed no anaphylaxis symptoms to oral peanut challenge compared with 80% of mice with anaphylaxis in the sham treatment group.³⁹ Furthermore, FAHF-1-treated mice had significantly lower histamine levels, reduced peanut specific IgE, and significantly less IL-4, IL-5, and IL-13 secreted from their splenocytes when compared with sham-treated mice.

The removal of 2 herbs—Zhi Fu Zi and Xi Xin—from FAHF-1 (based on presumed lack of significant effect and potential for toxic effects if incorrectly processed or overdosed) resulted in the creation of FAHF-2. The results in peanut sensitized mice were largely similar to the previous study in regard to reduction of anaphylaxis.⁴⁰ Levels of plasma histamine, peanut specific IgE, and T_H2 cytokines were significantly reduced compared with sham-treated mice, whereas the IFN- γ level was significantly elevated. Safety was demonstrated by feeding mice with 24 times the effective daily dose of FAHF-2 without any deaths and normal biochemical and major organ analyses. It was further discovered that FAHF-2 had long-lasting effects—reduced anaphylaxis scores and peanut specific IgE remained relatively constant 36 weeks after therapy or approximately 25% of the mouse life span.⁴¹ A phase 1 study in 18 humans completing the randomized, double-blind, placebo-controlled dose escalation trial showed that FAHF-2 was well tolerated during 7 days of therapy and only minor gastrointestinal symptoms were reported at a rate equivalent to those receiving placebo. There was a reduction in IL-5 after active treatment, but levels of IFN- γ , IL-10, and transforming growth factor β were unchanged in either treatment group.⁴²

Cellular Mediators

Platelet-activating factor (PAF) is a proinflammatory phospholipid released by mast cells during anaphylaxis, which leads to mobilization of calcium, vasodilation, and platelet aggregation.⁴³ PAF is regulated in the circulation by the enzyme PAF acetylhydrolase (PAFA). Vadas et al⁴⁴ studied PAF and PAFA levels in 41 patients with nonfatal anaphylaxis and compared them to 9 patients with fatal anaphylaxis to peanuts and 5 other groups: nonallergic adults, nonallergic children, healthy children with mild peanut allergy, patients with nonfatal acute anaphylaxis to peanuts, and children who died of causes unrelated to anaphylaxis. Fatal peanut anaphy-

laxis patients were found to have a significantly lower level of PAF compared with the other groups, and increased PAF levels correlated with higher anaphylaxis severity scores, suggesting it was an independent risk factor for fatal anaphylaxis. Use of a PAF receptor antagonist in a peanut-sensitized murine model significantly lessened the severity and length of anaphylaxis compared with those treated with antihistamines and untreated mice.⁴³ The combination of the antagonist with antihistamines further improved the results.

IL-12 promotes T_H1 cell differentiation and production of IFN- γ and inhibits T_H2 cell differentiation from naive T_H cells. Oral IL-12 administration to peanut sensitized mice was found to reverse and inhibit specific IgE synthesis and, therefore, reduce anaphylaxis severity.⁴⁵ IFN- γ levels were higher compared with sham-treated mice. Rather than primary therapy options, IL-12 or PAF receptor antagonists are likely to be reserved for use as an adjunct early on to reduce risk for anaphylaxis during escalation phases for immunotherapy.

Engineered Allergen Immunotherapy

Peptide immunotherapy uses allergen proteins separated into overlapping peptide fragments, 10 to 20 amino acids in length, that are unable to cross-link IgE antibodies on mast cells but still contain T-cell-stimulating epitopes. Early work on this form of immunotherapy was performed with cat allergens, but research in peanut allergy led to the discovery that pepsin-digested peanut contained T-cell epitopes but no IgE epitopes while inducing IFN- γ production.⁴⁶ The T-cell epitopes of major peanut allergen Ara h 2 have been mapped,⁴⁷ but epitopes of the other major peanut allergens have yet to be fully elucidated. This strategy could allow for higher doses of immunotherapy to be administered without risk of anaphylaxis, but the complexity of configuring hundreds of peptides into a single vaccine has largely led to abandonment of this therapy.

Elimination of IgE binding through alteration of the IgE-binding epitopes describes the strategy of mutated protein immunotherapy. Specific IgE binding in food allergy occurs to the linear epitopes rather than conformational epitopes as in the aeroallergens. This method of immunotherapy leaves the T-cell epitopes intact. Single mutations for alanine were encoded into 4 IgE-binding epitopes on the Ara h 2 gene and resulted in a decrease in binding when compared with the wild-type controls in 12 of 16 peanut-sensitive patients.⁴⁸ Similarly, 4 alanine substitutions in the 40-kDa subunit of the primary sequence of Ara h 3 resulted in substantial decreases in IgE binding, whereas stimulation of T-cell proliferation remained unchanged.⁴⁹

Bacterial Adjuvants

Increasing the effects of mutated protein immunotherapy through the use of bacterial adjuvants has been the focus of several studies involving heat-killed *Listeria monocytogenes* and heat-killed *Escherichia coli*. Early work by Frick et al⁵⁰ studied the effects of vaccination of peanut allergic dogs with heat-killed *L. monocytogenes* and peanut allergen and found

significant reductions in skin test reactivity, significant increases in tolerated doses on oral peanut challenge, and disappearance of IgE specific for Ara h 1 on immunoblot analysis. Furthermore, the effects of a single vaccination dose lasted at least 5 months. Use of subcutaneous heat-killed *L. monocytogenes* and the modified peanut proteins (mAra h 1–3) in a peanut sensitized mouse model revealed a marked reduction in incidence and severity of peanut-induced anaphylaxis compared with sham-treated mice or those treated with mAra h 1–3 alone.⁵¹ Cytokine analysis demonstrated a shift from T_H2 toward a T_H1 profile.

The engineered proteins used in the previous study were produced in *E. coli*; therefore, it was thought that heat-killed *E. coli* may serve as an effective adjuvant itself when combined with mAra h 1–3. Preliminary testing in mice with subcutaneous and rectal routes of administration demonstrated that both suppressed peanut allergy, but the subcutaneous route led to skin inflammation and would likely pose an infectious risk if used in humans. Therefore, subsequent testing focused on the rectal administration of heat-killed *E. coli*–mAra h 1–3 in peanut sensitized mice.⁵² Three log¹⁰ dose difference groups of mice were treated at 10 weeks after sensitization and compared with sham, heat-killed *E. coli* vector alone, and naive mouse groups during peanut challenges at weeks 14, 18, and 22. Only the medium- and high-dose heat-killed *E. coli*–mAra h 1–3 groups had significant reductions in anaphylaxis symptom scores at weeks 18 and 22, and this effect lasted at least 10 weeks after therapy. Currently, a product for humans, EMP-123, is in phase 1 clinical trials.

Plasmid DNA Immunotherapy

The immunization of plasmid DNA (pDNA) encoding an antigen leads to the production of the antigen protein after uptake by antigen-presenting cells and, as a result, leads to both cellular (T_H1) and humoral immune responses to that antigen. The varying responses across strains of mice have raised concerns for the potential application of pDNA immunotherapy in humans. In a study of mice receiving intramuscular injections of pDNA encoding Ara h 2 followed by peanut challenge 3 weeks later, AKR and BALB/c mice species were not sensitized to peanut, whereas C3H mice were sensitized and experienced severe anaphylaxis, with worsened severity (60% mortality) correlating to multiple injections.⁵³ Separately, oral immunization with chitosan-DNA for Ara h 2 reduced anaphylaxis severity and peanut specific IgE levels after peanut challenge in sensitized AKR mice.⁵⁴

Immunostimulatory Sequence and Oligodeoxynucleotide-Based Immunotherapy

Immunostimulatory sequence (ISS) and oligodeoxynucleotide-based immunotherapy incorporates pDNA conjugated with synthetic ISSs containing unmethylated cytosine and guanine dinucleotide repeat motifs. These guanine dinucleotide motifs induce a T_H1 response through binding to Toll-like

Table 2. Therapies, Mechanisms, and Clinical Utility for Food Allergy

Therapy	Route	Human studies	Mechanism	Comments
Oral/sublingual (see Table 1)	Oral	Yes	Desensitization demonstrated, T _H 2 cytokine reduction and increase in T-regulatory function, long-term induction of tolerance unknown	Lower risk of anaphylaxis compared with injections
Anti-IgE antibodies ^{32,33}	Subcutaneous	Yes	Inhibits IgE binding to high-affinity IgE receptor, downregulates production of IgE receptor, inhibition of mast cell degranulation	Lacks uniformity in protection
Probiotics ³⁴⁻³⁶	Oral	Yes	Potential increase in IgA, ex vivo results show decreased TNF- α and IL-10	Prolonged therapy revealed no change in peanut specific IgE or SPT wheal sizes, limited effectiveness demonstrated in atopic dermatitis
Soy ³⁷	Intraperitoneal	No	Uses homologous proteins, lowers peanut specific IgG1, IgG2, and total IgE, raises IFN- γ and TNF- α	Intraperitoneal sensitization and desensitization clinically impractical, caution in concomitant soy allergy
Chinese herbal medicine ³⁸⁻⁴²	Oral	Ongoing (Phase I completed)	Unknown mechanism, reduction in T _H 2 cytokines and peanut specific IgE, increased IFN- γ	Short-term safety demonstrated in phase 1 trials, animal studies show long-term protection, potential for application to other food allergens
Cellular mediators PAF receptor antagonists ^{43,44}	Oral	No	Blocks the effects of PAF, which is released from mast cells during anaphylaxis	Combination with antihistamines most effective, potential use for acute prevention before challenge/induction of desensitization
IL-12 ⁴⁵	Oral	No	Promotes T _H 1 cell differentiation and IFN- γ upregulation, inhibits T _H 2 cell creation	Preventive and therapeutic potential, utility possibly limited by its short half-life
Engineered allergens Peptide immunotherapy ^{46,47}	Subcutaneous	No	Overlapping peptides are unable to cross-link IgE antibodies on mast cells, T-cell stimulation maintained, increased IFN- γ production	Potential for higher doses of immunotherapy, likely improved safety, expensive to configure various proteins into single vaccine
Mutated protein ^{48,49}	Subcutaneous, intranasal, and oral	No	Reduced IgE binding through alteration of IgE binding epitopes, T-cell response maintained	Improved safety, identification of IgE-binding epitopes required, human studies planned
Bacterial adjuvants HKLM ^{50,51}	Subcutaneous	No	Enhanced innate and adaptive immune response by adjuvant stimulation of pathogen-associated molecular patterns, cytokine shift towards T _H 1 profile	Concern for infectious risk of bacterial adjuvants, long-lasting protection
HKE ⁵²	Subcutaneous and rectal	In Phase I trials currently		Rectal administration regarded as safest form, protection in mice lasted 10 weeks
Plasmid DNA ^{53,54}	Subcutaneous and oral	No	Antigen production via integration into DNA, induces T _H 1 and humoral responses to antigen	Strain-dependent response causing serious concerns for safety, possibility of single-dose therapy, possible excess T _H 1 stimulation
Immunostimulatory sequences ⁵⁶	Subcutaneous	No	CpG motifs promote T _H 1 response via TLR9 binding on macrophages and dendritic cells, production of IFN- γ and IL-12	Potential for prevention of food allergy, therapeutic ability not studied, possible excess T _H 1 stimulation

Abbreviations: HKE, heat-killed *Escherichia coli*; HKLM, heat-killed *Listeria monocytogenes*; IFN- γ , interferon γ ; IL, interleukin; PAF, platelet-activating factor; SPT, skin prick test; TLR9, Toll-like receptor 9; TNF- α , tumor necrosis factor α .

receptor 9 on macrophages and dendritic cells and subsequently stimulating production of IFN- γ and IL-12.⁵⁵ ISS–Ara h 2 intradermal immunization was studied in mice who were sensitized to peanut intragastrically 1 month after injection and then challenged to Ara h 2 after 5 weeks.⁵⁶ The ISS–Ara h 2 treated mice did not develop anaphylactic symptoms and had significantly reduced plasma histamine levels compared with controls in this unpublished study. ISS and pDNA therapies have shown potential for prevention of sensitization, but the ability to reverse food allergy has remained unstudied. Table 2 provides a summary of the therapies, mechanisms, and clinical utility for food allergy.

CONCLUSION

Many of the preceding therapies have shown some potential for either prevention or treatment of peanut allergy, yet the clinical application of these results is uncertain. Widespread availability of peanut flour, whole peanut extract, and commercial anti-IgE antibodies makes these the most likely options for use by the allergist. However, our eagerness to help the patient must be tempered by our primary objective to not harm those within our care.

As much as we know regarding food allergy and the mechanisms of these novel therapies, more striking is the number of questions that have yet to be answered. Specifically, questions regarding patient selection, optimum dosage and collaborated dosing schedules, duration of therapy, protection after therapy discontinuation, and even definitive tolerance vs desensitization still remain ambiguous and enigmatic. Although peanut OIT is relatively safe, it is not without risk, and that risk must be reconciled with the individuals' willingness to undergo therapy to alter their condition at the expense of a large amount of time, resources, and health care dollars. Therefore, adoption of these therapies to treat peanut allergy must currently be strongly discouraged in the clinical setting. However, we can look forward with excited anticipation as the answers to these questions are discovered in the years ahead.

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