

Current and emerging immunotherapeutic approaches to treat and prevent peanut allergy

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Peanut-allergen hypersensitivity reactions, which can result in anaphylactic episodes and death, affect approximately 1% of the general population. Currently, strict avoidance of allergenic food is the only available treatment for this food-induced allergic reaction; however, the innocuous presence of trace amounts of peanut protein contaminating food products makes avoidance extremely difficult, especially in children. Therefore, safe and inexpensive therapeutic strategies aimed at prevention and treatment of peanut allergies is urgently required. This review summarizes the current state of knowledge of adaptive immune recognition and responsiveness to peanut allergens and how this can be integrated and subverted into new therapeutic treatment regimens for these dangerous allergic responses. The potential for new strategic vaccination-based interventions to either moderate or prevent these types of responses from occurring is also discussed.

KEYWORDS: anaphylaxis • hypersensitivity • immunoprophylaxis • peanut allergens • vaccination

The prevalence & natural history of peanut allergy

Food allergy accounts for about 30,000 anaphylactic reactions, 2000 hospitalizations and 200 deaths each year in the US alone [1]; if, as predicted, 5% of the child population of the UK and Ireland are similarly afflicted then it has been predicted that upwards of 1 in 800,000 food allergic children will die each year [2]. Peanuts are the major causative allergen with an incidence of approximately 1% of the US population [1], a statistic also recapitulated in Europe [1,3–5]. The incidence of peanut allergy in children has shown a continued upward trajectory during the past two decades. The first evidence for this was noted from a study conducted in American children with atopic dermatitis from 1990 to 1994. Here it was concluded that the prevalence of peanut allergy had doubled from that observed in a similar group of children 10 years earlier [6]. In other long-term follow-up studies, it was shown that in the general US population, the relative incidence of peanut and/or tree nut allergies had changed little in years from 1997 (1.4%) [7] to 2002 (1.2%) [8] to

2008 (1.4%) [9]. Although the adult prevalence of peanut allergy, tree nut allergy or both was similar (1.3%) in both 1997 and 2002 [7,8], the allergy rates of children (under 18 years of age) increased markedly over the same time period *viz.*, 0.6% (1997) [7], 1.2% (2002) [8] and 2.1% (2008) [9].

The basis for the observed increase in peanut allergy among children is poorly understood. However, as vegetarian diets become more popular and as the demand for preprepared nutritional foods grows, peanut exposure has increased in pregnant and nursing mothers and in infants [10,11]. Consequently, virtually all children in the USA are exposed to peanuts by the age of two [12]. Owing to the relative immaturity of the infant immune system, immunoreactivity to food is more likely to develop during the first few years of life. Importantly, increased exposure throughout childhood translates into increased peanut sensitization in genetically predisposed children [13].

The initial reaction to peanuts typically occurs at a median age of 14–24 months [14,15], and unlike milk and egg allergies, most (but not

all [16]) peanut-allergic patients do not outgrow their peanut sensitivities [10]. For the majority of patients who continue to have clinical sensitivities to peanuts, reactions may become more severe with time [10]. Many studies have similarly shown that 72–81% of peanut-allergic patients react to peanuts on their first known ingestion and the severity of subsequent responses is more often than not similar [14,17], but up to a third of the patients with mild initial reactions develop severe symptoms on subsequent exposures [17].

Clinically relevant peanut allergens

In general, food allergens are low molecular proteins or glycoproteins (less than 70 kD) that are soluble in aqueous solution and abundant in the food source. These molecules are generally resistant to proteases, heat and denaturants, thus preventing destruction during food preparation and digestion [18,19]. Eleven peanut allergens (Ara h 1–11) have been described in the literature, all of which have been sequenced and cloned [20]. These allergens have demonstrated either clinical reactivity in peanut allergic patients or are associated with reactivity to peanut allergy patient sera (TABLE 1). Ara h 1 was the first major peanut allergen identified [21], and has an average molecular weight of 63.5 kD and an isoelectric point of 4.55. The gene for Ara h 1 has significant sequence homology with plant seed storage proteins of the vicilin family [22]. The second major peanut allergen characterized was Ara h 2 [23], which has a molecular weight of 17.5 kD, an isoelectric point of 5.2 and a 20% carbohydrate content. The sequence of the Ara h 2 gene revealed significant nucleotide homology with the conglutin seed storage protein family. Ara h 3, a member of the glycinin family of seed storage proteins, was the third allergen identified [24] and was found to bind to peanut-specific serum IgE in almost half of the peanut-allergic patient population [25]. Ara h

4 is highly homologous in amino acid sequence to Ara h 3 and has since been renamed Ara h 3.02 [201]. Ara h 5 is a member of the profilin family [26]. Ara h 6 and h 7 are also members of the conglutin family [26], and are thought to be various isoforms of Ara h 2. Ara h 6 has recently emerged as a major peanut allergen [27]. Ara h 8 is a major allergen in patients with both birch pollen and peanut allergy [28]. Ara h 9 is a newly discovered peanut allergen with clinical relevance for the Mediterranean allergic population [29]. Ara h 10 and Ara h 11 are also recently described isolates that have demonstrated some immunoreactivity against allergic patient sera and could potentially emerge as important peanut-associated allergens.

The three major allergenic proteins in peanuts, Ara h 1, Ara h 2 and Ara h 3, share common characteristics with many other allergenic food proteins: present in high concentrations, water soluble, and resistant to heat and digestion [30]. Although peanuts belong to the legume family, less than 15% of peanut-allergic patients react to proteins from other members of the legume family [31]. Interestingly though, tree nuts belong to a different botanical family, and yet 25–35% of patients who are allergic to peanuts also have allergic reactions to tree nuts [10]. The most common tree nuts that cause allergic reactions are Brazil nuts, almonds, hazelnuts, walnuts, almonds, pecans and cashew nuts [14,32,33].

Risk factors for peanut allergy

IgE-mediated peanut allergy is a complex trait with strong heritability but its genetic basis is currently unknown. Loss-of-function mutations within the filaggrin gene are associated with atopic dermatitis and other atopic diseases; therefore, filaggrin is a candidate gene in the etiology of peanut allergy [34]. Suggested potential risk factors for the development of peanut allergy include atopy, family history of peanut allergy and prenatal maternal consumption of

Table 1. Clinically relevant peanut (*Arachis hypogaea*) allergens.

Allergen	Homology	Molecular weight (kDa)	Nucleotide length (bp)	IgE reactivity (%) (allergic patients)	Genbank accession number	Ref.
Ara h 1	Cupin (vicillin-type)	64	2032	88	L34402.1	[54]
Ara h 2	Conglutin	17	472	85	AY007229, AY158467	[24]
Ara h 3	Cupin (legume-type)	60, 37	1524	44	AF093541.1	[20]
Ara h 3.02	Cupin (legume-type)	61	1853	44	AF086821	[20,201]
Ara h 5	Profilin	15	743	13	AF059616.1	[24]
Ara h 6	Conglutin	15	627	38	AF092846	[24]
Ara h 7	Conglutin	15	712,495	43	AF091737, EU046325	[24]
Ara h 8	Pathogenesis-related protein	17	474,462	85	AY328088, EF436550	[26]
Ara h 9	Nonspecific lipid-transfer protein 1	9.8	351,271	Minor allergen	EU159429, EU161278	[27]
Ara h 10	Oleosin	16	954,578	7 patients	AY722694, AY722695	[201]
Ara h 11	Oleosin	14	655	7 patients	DQ097716	[201]

peanuts [11,35]. Other suggested risk factors include infant consumption of peanuts and peanut oil [36], and use of maternal breast creams containing peanut oil [37]. The consumption of soy milk or formula by infants was also implicated in early studies [38], but more recent findings strongly suggest otherwise [39,40]. Recently, it was reported that the formation of Maillard reaction products by heating the major peanut allergens in the presence of sugars enhanced the allergenicity of Ara h 2 by increasing IgE binding capacity, presumably by exposing otherwise hidden immune-reactive moieties [41]. Interestingly, the overall incidence of peanut allergy is lower in Asian countries [42]. One possible explanation for this lower incidence is the method of food preparation. In Asian countries, peanuts are often eaten raw or boiled rather than fried in oil or roasted as is common in Western countries. Indeed the levels of Ara h 1, a major peanut allergen, are up to 22-fold higher in roasted than in raw peanuts [43].

Diagnosis of peanut allergy

A previous clinical history combined with open food challenge has been reported to provide a good predictive value; however, the primary clinical tool used to evaluate the food-allergic patient is skin-prick testing with different food sources, which can result in an immediate wheal and flare reaction. When skin-prick testing showing a wheal ≥ 16 mm was combined with peanut-specific IgE antibody levels ≥ 57 kU of antibody/l, a diagnosis of peanut allergy could be made with specificity and positive predictive values both of 100% [44]. Despite the utility of this approach, in the rare instances where patients reach this very high cut-off level serum of peanut-specific IgE antibody, it remains that the placebo-controlled, double-blind food challenge is still considered the 'gold standard' for the diagnosis of peanut-related allergy [45], despite the obvious potential risks associated with its use, which naturally limits its application in the clinic.

The immunopathogenesis of peanut allergy

Allergic diseases result from aberrant immune responses directed against otherwise harmless environmental antigens. Allergic diseases, which include asthma [46], allergic conjunctivitis [47], peanut allergy and anaphylaxis [48], are centered around type 2 immune responses, which are also used by a host to control parasitic infections. Conversely, type 1 immune responses are characterized by antibodies predominately of IgG2a isotype in the mouse, activation of NK cells and phagocytic cells, and the development of cytotoxic T lymphocytes (CTL). Type 1 immunity is mainly used by a host to defend against viral and intracellular bacterial infections. Both type 1 and 2 responses are coordinated by helper T cells, which differentiate into several functionally different subsets including T_H1 and T_H2 lymphocytes. These subsets are characterized by their cytokine secretion profile [49], where T_H1 cells produce IFN- γ and T_H2 cells typically secrete IL-4, IL-5 and IL-13. The differentiation of T_H1 and T_H2 cells is a multifactorial process but by far the most influential aspect is the cytokine milieu present at the time of initial T cell activation [50,51]. While IL-12 facilitates differentiation toward a T_H1 type immune response, IL-4 allows for T_H2 differentiation; these

cytokines maintain and stabilize the balance of the T_H1 and T_H2 responses. IFN- γ promotes T_H1 cell development by stimulating IL-12 production by macrophages and dendritic cells (DCs), and IL-12 receptor expression on $CD4^+$ T cells, while concurrently suppressing T_H2 cell differentiation. In contrast IL-4 and IL-13 partially inhibit IL-12 production and, as a consequence, suppresses the development of T_H1 cells. T_H2 subsets direct immunoglobulin class switching to IgG1 and IgE in mice (IgG4 and IgE in humans) and ultimately induces peanut allergy associated IgE-dependent and eosinophil/mast cell-dependent immunity.

Oral allergens, such as those derived from peanuts, first encounter the gut mucosal immune system. Microfold (M) cells are specialized follicle-associated cells that line the epithelium of the gastrointestinal tract and lie in close proximity to Peyer's patches. They are responsible for the induction of tolerising and/or protective gut-associated immune responses. Sensitization to food allergens occurs when exogenous food antigens are taken up by M cells, and then presented to macrophages and DCs [52]. Once internalized by macrophages and DCs, the antigens are endocytosed, then denatured and degraded into peptides of 12–20 amino acids in length. A minute fraction of these small peptide fragments are then transported intracellularly and presented on cell-surface MHC class II molecules for specific interaction with $CD4^+$ T cells. These (now activated) T cells subsequently expand in number and release T_H2 cytokines. The T_H2 T cells, IL-4 and IL-5, promote the differentiation of B cells, which bear allergens bound to surface Ig receptors, into cells that secrete allergen-specific IgE antibodies [53]. These IgE-producing B cells then expand in number and become plasma cells with the sole function of continuously secreting allergen-specific IgE antibodies. Environmental exposure to peanuts results in binding of peanut allergens to specific IgE-coating mast cells and basophils. Subsequently, Fc receptor cross-linking provides a potent activation stimulus that results in the degranulation of basophils and mast cells. The rapid release of a variety of preformed proinflammatory and vasoactive compounds such as prostaglandins, leukotrienes, serine proteases, histamine and cytokines into the extracellular fluid ensues to produce inflammatory responses [54], all of which culminate in the clinical features of an acute allergic reaction [30]. Local symptoms of peanut allergy include abdominal pain, vomiting, cramping and diarrhea, and are common even in cases of mild peanut allergy. This acute nonlife threatening reaction causes a transient increase in intestinal permeability, which subsequently allows systemic distribution of macromolecules, such as whole peanut allergens, exacerbating any resulting immune responses [55].

The clinical manifestations & management of peanut allergy

Patients with allergic reactions to peanuts can present with a variety of symptoms. The early symptoms of anaphylaxis often include oral and pharyngeal pruritus, flushing, urticaria, nausea, vomiting and chest tightness. Progressive symptoms of diffuse wheezing, hypotension, arrhythmia and loss of consciousness can develop in near-fatal or fatal reactions [56]. Many peanut allergic

patients develop severe symptoms on exposure and only minute quantities can induce an anaphylactic reaction. Peanuts and tree nuts account for most of these reactions and cause between 40 and 80% of the deaths [57,58]. Risk factors contributing to fatal reactions were denial of symptoms, concomitant asthma and, most importantly, delaying administration of epinephrine [3,5,10,45,57].

The general management of peanut allergy is based on the following three approaches: strict avoidance of peanuts, prompt recognition of allergic reactions and rapid initiation of treatment for anaphylaxis [10]. Avoidance involves checking food product ingredient labels and avoiding high-risk foods such as unlabelled ice creams, cookies and candies. Similarly, high-risk locations where accidental peanut ingestion is more likely to occur include buffets and restaurants (crosscontamination of food during cooking), friends' houses and ice cream parlors, as well as day care centers and school cafeterias (food sharing). Nonetheless, given the wide and frequently hidden use of peanuts in food, avoidance can be problematical; it has been estimated that the average patient with an allergy to peanuts will have an allergic reaction from accidental ingestion of peanut protein every 3–5 years [14]. The definitive emergency treatment for anaphylaxis is the early administration of epinephrine and all patients at risk for experiencing anaphylaxis need to carry selfinjectable epinephrine. Failure to recognize the symptoms of anaphylaxis and failure to administer epinephrine immediately after the onset of anaphylaxis increase the risk of a fatal outcome [5,59]. The only effective treatment for anaphylaxis is treatment with intramuscular epinephrine, while oxygen, nebulized albuterol, systemic corticosteroids, and H_1 and/or H_2 receptor antagonists can also help alleviate secondary symptoms. Patients remain under medical supervision for at least 4 h to monitor for delayed reactions, and upon discharge a short course of oral prednisone and an antihistamine is also often given.

Immunotherapeutic approaches to peanut allergy

Immune deviation strategies

The defining immunopathologic feature of peanut allergy is the presence of circulating peanut protein-specific IgE antibodies in the susceptible individual. Therefore, any approach that reduces the levels of IgE has the potential to reduce allergic sensitivity. However, unlike traditional immunotherapy for allergic reactions to inhalants such as grass pollens, dust mite and bee sting venom, subcutaneous desensitization injections of peanut extracts have an unacceptable risk–benefit ratio [60].

Another approach may be to reprogram the immune response in a T_H1 or cell-mediated direction. Cell-mediated immunity is usually elicited in response to endogenous peptides, which for example may be derived from proteins synthesised within cells during a viral infection. Here, a finite amount of newly synthesised endogenous protein is degraded within the cell by the proteasome to produce 8–12mer peptides, which are delivered into the endoplasmic reticulum (ER) where they associate with MHC class I molecules on their way to the cell surface. $CD8^+$ T lymphocytes recognize the cell-surface MHC I/peptide complexes and release T_H1 cytokines, which mature $CD8^+$ T lymphocytes into CTLs [61]. Activated CTLs subsequently recognize cells

bearing cognate MHC I/peptide complexes and target them for destruction.

Exogenous proteins, which usually enter the MHC class II processing pathway, can be cross presented on MHC class I molecules by entering the MHC class I processing pathway, but only by immature DCs, which is a unique and highly specialized APC [62–65]. Simultaneous presentation via MHC I and MHC II pathways will activate $CD8^+$ and $CD4^+$ T cells to secrete T_H1 cytokines that will stimulate B cells to produce and secrete antigen-specific IgG2 antibodies. Because they do not bind to mast cells or basophils, these types of antibodies play no role in the induction of an allergic reaction although they do bind and neutralize pathogens and toxins, thus clearing them from the host.

In contrast, food allergens are presented to the immune system as exogenous peptides. Hence, in order to generate a T_H1 -biased immune response to food allergens, immunization protocols must aim at intracellular delivery of the allergen, so that entry into the MHC class I processing can occur directly or indirectly via cross presentation. Consequently, T_H1 -directed switching to IgG class might neutralize peanut proteins and suppress peanut-specific IgE antibody levels. Together, this maneuver has the potential to provide protection in those individuals predisposed to peanut allergy (i.e., those with family history of peanut allergy or known atopic individuals) and, importantly, a therapeutic immune response in those with pre-existing peanut allergy.

DNA immunization

Immunization with plasmid DNA encoding food allergens is a good example of this kind of approach of targeting antigen to the MHC class I processing pathway. The DNA vaccine is taken up by APCs where the DNA encoding allergen is transcribed, translated, and presented by MHC class I molecules to the APC surface to T cells. This endogenously produced allergic protein or protein fragment induces a T_H1 phenotypic response with up-regulation of T_H1 cytokines, an increase in IgG2 antibodies and suppression of allergen specific IgE antibody production [66,67].

The concept that immunizing with DNA coding for allergens might present a new approach to immunotherapy for allergic diseases was first described over 15 years ago [68], and the first practical example of this approach, which demonstrated alleviated responses, was to the house dust mite allergen Der p5 [69]. Here it was shown that in rats immunized with plasmid DNA encoding Der p5, that sustained antigen expression was evident and that it induced antigen-specific immune responses that inhibited IgE production and histamine release in bronchio-alveolar fluid. Airway hyper-responsiveness in rats challenged with aerosolized allergen was also suppressed and this suppression was persistent; when $CD8^+$ T cells from these immunized rats were transferred into naive rats, IgE synthesis was inhibited.

Another DNA immunization technique under investigation is immunization with plasmid DNA that coexpress to immunostimulatory sequences. These stimulate APCs and natural killer cells to secrete T_H1 cytokines that promote immune deviation toward the T_H1 phenotype and away from the allergic T_H2 phenotype [70]. Most of these DNA immunization techniques

have been studied in murine models. However, in a seminal study involving mice and rabbits, as well as in primates, it was shown that immune deviation was toward a T_H1 phenotype and a reduction in allergenicity was possible after injection immunotherapy with the major ragweed allergen, Amb a 1, conjugated to immunostimulatory sequences [71].

The high amount of plasmid DNA necessary for vaccination still remains the major shortcoming of this approach for allergy treatment in humans and clinical trials with DNA vaccines indicate poor immunogenicity of the constructs in humans [72]. However, the use of live viral vaccine vectors for delivery and expression of antigens in infectious diseases and cancer vaccination studies are starting to show promise [73–75], and this approach has potential applications in peanut immunotherapy.

Novel therapeutic strategies

Mutated peanut protein substitutes

Rather than avoiding peanuts, this approach enables the consumption of a 'defanged' food source. The primary amino acid sequences of IgE-binding epitopes of the major allergens present in foods are altered to reduce specific IgE binding [76]. This is possible because the specific IgE antibody binding to food allergens occurs to linear amino acid sequences in contrast to the binding of IgE antibody to inhaled allergens, which typically have conformational epitopes [77]. This technique has been shown to substantially decrease or inhibit IgE antibody binding with major peanut allergens. The major peanut allergens, Ara h 1, Ara h 2 and Ara h 3, have been mutated to make their complementary DNA, from which the mutated hypoallergenic proteins can be made. The major T-cell epitopes were not altered by mutating the IgE-binding sites. Peanut allergic patients had significantly reduced peanut-specific IgE binding to the mutated peanut protein compared with the wild-type peanut protein [78], and it is possible that future peanut crops could be grown with the mutated sequences, resulting in a hypoallergenic food product and hence allowing consumption in otherwise allergic individuals. Nonetheless, this approach has the disadvantage of being bypassed if a peanut-allergic individual is exposed to wild-type peanut proteins and, hence, conceptually is little different to peanut avoidance.

Another approach directly confronts the question of avoidance by proposing early exposure of the immature infant immune system to peanuts. The Learning Early About Peanut (LEAP) ([202] identifier: NCT00329784) clinical trial has enrolled approximately 640 children between 4 and 10 months of age and is designed to evaluate whether or not early exposure to peanuts in children with eczema or food allergy (high risk of developing peanut allergy) can promote immunological tolerance to these proteins. Assessment of tolerance or reactogenicity will be completed at 5 years of age to determine which works best in inducing tolerance to peanut protein, avoidance or consumption? The results of this study have the potential to drive future recommendations on peanut exposure. The trial is due for completion in 2013.

Humanized monoclonal anti-IgE antibodies

This approach accepts that peanut exposure is unavoidable and seeks instead to neutralize the maladaptive IgE responses made to

food allergens. This tactic is being evaluated in Phase I and Phase II trials as monthly injections of humanized recombinant anti-IgE antibodies, which bind to freely-circulating IgE and not to mast cell-bound or basophil-bound IgE. Once circulating IgE antibodies are bound by anti-IgE, they cannot bind to specific high-affinity Fc receptors on mast cells and basophils. The complexes of anti-IgE bound to IgE are then cleared from the circulation. Thus, activation of allergic responses, at least to small amounts of peanut protein, can be prevented.

A randomized, double-blind, dose-ranging trial in 84 patients with peanut hypersensitivity treated with anti-IgE (Hu-901) has shown that patients who received the highest dose of anti-IgE for 4 months were able to tolerate increased doses of peanut on post-treatment food challenge compared with the pretreatment challenge [79]. Specifically, the threshold for clinical symptoms increased from 178 mg, approximately half of a peanut, to 2805 mg, an amount approximately equal to nine peanuts. This specific antibody preparation (Hu-901) was not selected for further clinical development but another humanized monoclonal anti-IgE (Omalizumab) has been approved for clinical testing in moderate to severe asthma. This same antibody was tested in a follow-up double-blind placebo-controlled clinical trial aimed at reducing the risk of peanut allergy in patients with moderate-to-severe asthma. Unfortunately this study was halted early owing to two severe allergic responses during the initial prescreening period, which therefore had nothing to do with administration of the antibody, and thus only 14 subjects reached the study's primary end point. The placebo group were tolerant to 4.07-times the baseline dose of peanut flour, while the omalizumab-treated group were able to tolerate 80.9-times the baseline of peanut protein after 24 weeks of antibody treatment (0.016 mg/kg/IgE every 4 weeks) [80]. Although the study did not meet its predetermined end point, future studies with IgE-immunotherapy may provide useful outcomes for patients with pre-existing peanut allergy. However, this approach does also raise the obvious question of treatment durability and related cost–benefit aspects of sustained use of these types of very expensive biotherapeutics.

Some approaches rely on nonconventional routes of delivery of peanut proteins in order to bypass the gut mucosal immune system and the inevitably vigorous responses it will mount to peanut allergens in susceptible individuals. These include epicutaneous immunotherapy (EPIT), peanut oral immunotherapy (peanut OIT), sublingual immunotherapy (SLIT), rectal immunotherapy and peptide immunotherapy.

Epicutaneous immunotherapy

Following promising results in mice [81], a clinical trial using EPIT is currently being conducted. EPIT Epicutaneous Immunotherapy in Peanut Allergy in Children (ARACHILD; clinicaltrials.gov identifier: NCT01197053 [202]) is a double-blind, placebo-controlled food challenge clinical trial in children 5–17 years of age using an epicutaneous delivery system named Viaskin®. The novelty of the device is in its ability to deliver peanut allergens only to the epidermis of the skin without damaging the dermal layers and thereby avoiding direct contact with the bloodstream.

Children will receive the EPIT or placebo control daily for 6 months and then a double-blind placebo-controlled food challenge (DBPCFC) will be conducted every 6 months. The primary outcome measure is the proportion of patients able to consume >1 g of peanut protein symptom-free or a tenfold increase in tolerability over base line.

Peanut OIT

The use of oral immunotherapy for peanut allergy appears superficially to be counter-intuitive given that ingestion of peanuts or peanut-containing products is the likely causative agent of the disease. However, peanut OIT has a manageable safety profile and unlike subcutaneous immunotherapy, few adverse reactions have been reported; despite the fact that patients often do react during increases in dosing, the reactions tend to be mild and controllable. In one OIT study, the incidence of adverse reactions were reported at 3.5% under a home dosing regimen [82]. Furthermore, in a meta-analysis of all clinical trials investigating the efficacy of OIT, approximately 50–75% of patients undergoing OIT tolerate the maintenance dose [83]. With such a low incidence of adverse reactions, the moderate success rate and the noninvasiveness of the treatment, several concurrent clinical trials are now in progress. For example, a 9-year study nearing completion ([202] identifier: NCT00598039) is assessing the ability of increasing doses of defatted peanut flour in 1–16 year-old children. Approximately 40 subjects are enrolled with the primary outcome measure being the subject's ability to pass a double-blind placebo–food challenge (DBPFC) after the tolerisation period and again 1 month after abstinence from peanuts. The completion date is late 2012.

Although the reasons for induced peanut tolerability and success of OIT are not fully understood, some interesting observations alluding to probable mechanisms have been described. The relative cellular make up of the oral immune system is thought to be pivotal. The mucosal presence of tolerogenic APCs known as Langerhans cells, which are involved in driving the immune response toward a T_H1 bias via secretion of cytokines such as IL-10 and IL-12 [84], combined with the relative absence of proinflammatory cells such as mast cells and eosinophils [85], establishes an environment primed for induction of oral tolerance.

Sublingual immunotherapy

SLIT is another exciting advance in the field of peanut immunotherapy. The procedure involves placing emulsified purified peanut protein under the tongue for 2 min and then swallowing. In one study, 4182 doses of SLIT were administered to 18 children (ages 1–12 years) over a 12-month period. Remarkably, only 0.26% of subjects required antihistamine for mild allergic symptoms and epinephrine treatment was not required. At the end of the study, a double-blind placebo-controlled food challenge was administered. The median peanut protein dose tolerated in the active arm was 170 mg (approximately 6–7 peanuts) while the placebo arm could only tolerate a median of 85 mg of peanut protein. Additionally, a significantly decreased skin test wheal was recorded on those subjects who underwent SLIT [86]. Consequently, another larger trial due for completion in 2014 is currently in progress. This trial

is a randomized, cross-over Phase I/II clinical trial of approximately 172–216 weeks duration in subjects aged 12–40 years of age receiving either a gradual dose escalation of peanut SLIT or placebo. The subjects will receive a series of six oral food challenges over a 44-week timeframe with maintenance therapy being continued for a further 2 years. The primary outcome measure is the percentage of desensitized participants while on daily SLIT, as measured by either tolerance to a 5-g oral food challenge or a tenfold increase in the amount of peanut powder (ClinicalTrials.gov identifier: NCT00580606) [202].

Rectal immunotherapy

An alternative form of mucosal immunotherapy currently being trialled involves heat-killed *Escherichia coli* expressing Ara h 1, 2 and 3 proteins. In a mouse model of peanut allergy, rectally-administered high (90 µg) and medium (9 µg) doses of the recombinant bacteria induced immune tolerance, thus preventing anaphylaxis reactions following peanut challenge for a period of up to 10 weeks [77]. Rectal delivery of antigens may be advantageous because *E. coli* and its expressed antigens are able to be delivered directly to the rectal mucosa as native proteins, resulting in enhanced presentation to the immune system. Consequently, the harsh and degradative milieu of the upper digestive tract is avoided. A Phase I clinical trial where capsules containing peanut protein are administered rectally is currently being performed to assess safety (ClinicalTrials.gov identifier: NCT00850668) [202].

Peptide immunotherapy

Peptide immunotherapy is a rapidly developing area within the field. The basis of this therapy is to target allergen reactive $CD4^+$ T cells and hence, eliminate or minimize downstream allergic responses. Several possible scenarios regarding the mode of action of peptide immunotherapy include, targeted deletion of $CD4^+$ T cells [87], causation of anergy in previously activated $CD4^+$ T cells [88] and/or the regulation of $CD4^+$ T cells by regulatory T-cells [89]. Typically though, peptides are selected and identified for immunotherapy applications by laborious methodologies, comprised of testing *ex vivo*-propagated $CD4^+$ T cell lines and clones from reactive individual in various assay formats for reactivity against pools of overlapping synthetic peptides with sequences derived from the corresponsing relevant antigens. However, more recently systematic approaches to this are being utilised, including peptide microarray platforms [90] and more readily available MHC class II tetramers [91], which may facilitate future epitope identification. Early work in the field of peanut allergy demonstrated that a series of overlapping peptides comprising T cell epitopes of peanut protein, reversed sensitivity to peanuts in a murine model of peanut-induced anaphylaxis, without triggering IgE-mediated acute reactions [92]. More recently, the mapping of the major T cell epitopes of Ara h 2 has produced a series of peptides suitable for targeting allergy specific-pathogenic T cells. Using overlapping, Ara h 2 peptide libraries and Ara h 2-specific $CD4^+$ T cells lines generated from peanut allergy patients, five immune-dominant Ara h 2 epitopes were discovered. Three short HLA diverse and

variant peptides that did not bind patient serum IgE but were still able to bind to pathogenic CD4⁺ T cells were tested [93]. These short immunotherapeutic-peptides mitigated the significant anaphylactic potential associated with low-dose administration with whole peanut proteins to peanut allergic patients, while theoretically maintaining the ability to induce a tolerising immune response. This is due to the fact that the peptides are unable to cross-link IgE antibodies and therefore unable to activate mast cells. Clinical trials are currently planned and if successful, could provide a new therapeutic avenue for peanut allergic individuals.

Chinese herbal medicine

Chinese physicians have for centuries known about the healing and therapeutic potential of various plants and animals. In respect to peanut allergy, a Chinese herbal medicine known as FAHF-2 demonstrated excellent results in a mouse peanut allergy model [94]. Mice were fed the compound twice daily throughout the model's sensitization period and then challenged with the peanut allergen. Remarkably, all mice tested were protected from anaphylactic responses and elicited significantly reduced serum IgE responses. This study has recently been followed by a mouse model of multiple food allergy (peanut, codfish and egg). Similarly, those mice fed FAHF-2 exhibited no anaphylactic reactions following challenge and also had reduced allergen-specific IgE levels [95]. Finally, a Phase I safety trial in humans was conducted, which concluded that FAHF-2 administration was a safe approach for prevention of allergies with only mild gastric irritation reported [96]. A Phase II safety/efficacy trial is currently enrolling participants.

Expert commentary

Peanut allergy is a frightening condition increasing in prevalence in which acute, life-threatening attacks can occur unpredictably. Even the sensible although burdensome strategy of allergen avoidance is not without risk since peanut contamination of modern processed foods is ominously common. Given the unpredictable nature of the attacks, any worthwhile immunotherapeutic approach must attenuate allergic reactions to trivial levels or eliminate the risk of serious reactions with certainty.

Principally, allergies are conditions of the immune system associated with a dysregulation of the T_H1 and T_H2 lymphocyte populations [97–99]. It has been proposed that vaccination and use of antibiotics, and increased sanitary living conditions, has resulted in a subsequent decline in occurrence of infectious diseases. Cocommittedly, T_H1 immune stimulation has also declined, with a resultant increase and in some cases detrimental shift toward a T_H2 bias of immune responses against normally innocuous environmental allergens, (i.e., hygiene hypothesis) [100,101]. An allergen vaccine that can activate a T_H1-directed immune response against the disease causing allergen offers a new approach for specific immunotherapy by redirecting the allergic T_H2 immune response.

The successes and tolerability in numerous past and ongoing clinical trials of oral immunotherapy places this treatment regimen as the lead candidate for the treatment of peanut allergy.

The recent discovery and full characterization of several new peanut allergens in combination with modern molecular and immunological tools has allowed for the synthesis of recombinant peanut proteins and peptides to be used in immunotherapeutic dosing protocols. For instance, the recent mapping of the Ara h 2 immunodominant epitopes and subsequent design of the HLA diverse and variant peptides that do not bind patient serum IgE but are able to bind to pathogenic CD4⁺ T cells, is a major advance in the field of peanut allergy. It is expected that similar testing will be performed in the near future for all the peanut allergens. In combination, these peptides may provide a simple but safe immune-modulating vaccine for peanut allergic individuals. The clinical trial named LEAP is a potentially ground-breaking study that has the potential to drive future recommendations regarding peanut consumption in children. The remarkable preclinical efficacy of FAHF-2 is suggestive of real potential but the active constituents need to be identified to ensure optimal formulation. Clearly though, the inclusion of several different immunotherapeutic modalities in combination may provide additional therapeutic benefits.

Five-year view

The incidence of peanut allergy has increased significantly over the previous decade and it therefore should be expected that the incidence will remain at current levels or higher. One key issue regarding peanut allergy is whether exposure to peanuts early in life increases or reduces the incidence of allergy. More definitive answers are anticipated following completion of the LEAP trial in 2014, which should provide medical practitioners and nutritionist's solid data for recommendations on management and prevention of peanut allergy.

Although only in early stages of development, peptide immunotherapy has the potential to provide patients with a simple but highly targeted approach toward modulating the immune responses to peanut allergy. The results of coming clinical trials are eagerly awaited and if successful will surely drive the impetus for continued mapping and identification of the major allergenic epitopes of the other peanut allergens.

Finally, the newer immunotherapeutic modalities including epicutaneous immunotherapy and in particular Chinese herbal preparations have shown promising preclinical and Phase I safety data. If similar safety and efficacy profiles of these treatments are successful in larger cohorts of peanut allergic individuals, the use of them either alone or in combination with traditional oral immunotherapy could represent a major paradigm shift in current treatment schedules.

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Key issues

- Peanut allergy is increasing in prevalence and causes unpredictable and life-threatening attacks.
- The identification and detailed characterization of the major peanut allergens is facilitating the development of new immunotherapeutic treatment regimens.
- Novel therapeutic approaches to treat or prevent peanut allergy are progressing through many different clinical trials.
- An increasingly mechanistic understanding as to how peanut allergy develops will also inform new therapeutic approaches to alleviate the condition, or better still, prevent it from ever occurring.
- It is anticipated that the outcomes of these ongoing clinical trials will change for the better the long-term prospects for those afflicted with peanut allergies.

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