Food allergy diagnosis and therapy: where are we now?

Food allergy is a growing worldwide epidemic that adversely effects up to 10% of the population. Causes and risk factors remain unclear and diagnostic methods are imprecise. There is currently no accepted treatment for food allergy. Therefore, there is an imminent need for greater understanding of food allergies, revised diagnostics and development of safe, effective therapies. Oral immunotherapy provides a particularly promising avenue, but is still highly experimental and not ready for clinical use.

KEYWORDS: allergy diagnosis ■ food allergy ■ immunotherapy ■ oral immunotherapy

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IgE-mediated food allergy (FA) is a growing problem worldwide. Defined as an immune response to a given food that occurs reproducibly upon exposure [1], FA affects anywhere from 1 to 10% of the population, with greater prevalence in children (4–6% in the USA vs ~2% in adults) [2]. This prevalence has been increasing at a rapid rate, as has been demonstrated by data from the USA [3], UK [4], Australia [5] and China [6]. Despite this burden, the only currently accepted treatment for FA is complete avoidance of the offending allergen, with epinephrine delivered in the case of accidental ingestions – which occur frequently, even in the most careful patients, and are often undertreated [7,8]. As such, FA is a highly stressful condition, generating elevated anxiety in allergic subjects and their families. FA is associated with significantly decreased quality-of-life scores to a degree that is greater than that seen in many other chronic childhood diseases [9,10]. There is, therefore, an urgent need for an effective therapy for the treatment of FA. Contingent with this is the need for greater understanding of the mechanisms of FA, as well as a need for more precise diagnostics. There is still much that remains unknown, and extensive research in many areas is needed to fully understand this disease and potential treatments. This article aims to address the current state of the field and to speculate on its future.

Pathophysiology & primary prevention

■ Pathophysiology

FA is currently regarded as a dysregulation of normal immune tolerance mechanisms. All food proteins are recognized as foreign antigens by the gut [11], but only allergic individuals demonstrate an immune response to these antigens. Tolerance, in normal individuals, is therefore believed to be a suppression of this response [2].

Development of FA is characterized by two stages:

- Sensitization, in which the allergic reaction pathways to the allergen are established;
- Elicitation, in which the immune system carries out an inflammatory response upon allergen re-exposure.

During sensitization, dendritic cells [12], the major class of APCs in the gut [13], present the allergen to T cells, stimulating the production of Th2 cytokines: IL-4, IL-5 and IL-13. These cytokines, in turn, stimulate B-cell class switching to produce the antibody IgE, which then binds to its high-affinity receptor on mast cells and basophils [2]. Cytokines and chemokines induce T and B cells, thus perpetuating the allergic response [13]. Acute symptoms include urticaria, flushing, angioedema, abdominal pain, nausea, vomiting, diarrhea, wheezing, coughing and/or bronchospasm, rhinorrhea and hypotension or syncope [2].

Not all foods are allergenic; in fact, of the over 12,000 food allergens known, only a small number induce allergies [7]. Further questions are raised by spontaneous resolution of FAs. Children usually outgrow allergies to milk, egg, soy and wheat, but not peanut or tree nut allergies [7]. Why this happens, and why only some FAs resolve independently while others remain, is unclear. Studies suggest IgE-binding patterns and, in various cases, IgE recognition of specific epitopes or amino acid sequences in peptides, may in some part be related to allergen severity and persistence [14].

The environment of the gut is also likely to be crucial. Intestinal permeability is positively

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associated with increased FA incidence; a study of food-allergic infants demonstrated they had greater intestinal permeability compared with healthy infants, an effect that lasted even after 6 months on an exclusion diet [15,16]. Likely to be even more important are the microbiota found in the gut. The hygiene hypothesis suggests that changes in the pattern of intestinal colonization during infancy and decreased exposure to infectious agents in childhood are important factors in the development of allergic disease, and may help explain why allergy prevalence is increasing [17,18]. Antibiotic use [19] and exposure to pets, farms and farm animals [20–23] have been linked to decreased atopy risk. In addition, differences have been found in gut microbial flora between allergic and nonallergic children [24,25], suggesting certain microbes may be more important to sensitization than others.

Work by Gupta *et al.* has provided considerable data regarding the current state of pediatric FA in the USA. A comprehensive survey of American children, published in 2011, found FA prevalence of 8%, with slightly higher prevalence found in children of Asian or African descent. A total of 38.7% of allergic children reported severe reactions, mostly to peanut, tree nut, shellfish, soy and fin fish. In addition, 2.4% of all children were found to have more than one FA, highlighting the need for greater general understanding of the disease and the development of multiple allergen therapies [26].

FA has a strong genetic component. Multiple studies have reported a strong family association [27], with one UK study finding a seven-fold increase in peanut allergy risk if an individual has a peanut-allergic parent or sibling [28]. A study in a Chicago cohort found varying heritabilities associated with different allergens [29]. A study of peanut allergy in twins found a much higher concordance for the allergy between identical twins than between fraternal twins (64.3 vs 6.8%, respectively), with an estimated heritability of 81.6% [30]. Studies have also found associations with race and ancestry [31].

Primary prevention

The American Academy of Pediatrics, in 2000, outlined heavy dietary restrictions for allergenic foods through breastfeeding and the first 3 years of life; however, recently, in the absence of sufficient data, has retracted most of these guidelines [32]. National Institute of Allergy and Infectious Diseases, too, holds that there are insufficient data to suggest maternal diet influences the development or course of FA in children [33]. There is also considerable debate regarding whether sensitization can occur *in utero* [28,34].

Several observational cohort studies have found that earlier introduction of foods may decrease allergy risk, and that delayed introduction of foods and/or extended breastfeeding may increase allergy risk [12,35–38]. These studies are still underway and we are awaiting further results from these randomized control trials. One particularly prominent study, and one of the first to look at early exposures in a large, geographically diverse cohort of subjects, was published by Lack's group in the UK in 2008, which compared Jewish children in the UK, who avoided peanuts for most of their first few years of life, with Jewish children in Israel, who were introduced to peanuts early during weaning and who continued to eat it more frequently and in greater amounts than children in the UK [38]. The study controlled for various factors including method of preparation, and the group found significantly lower rates of peanut allergy in Israeli Jewish children than British Jewish children. This provided evidence that early exposure to highly allergenic foods might be preventative for allergies to these foods; further research into this is ongoing [38].

Building on this idea, nutrition and diet have also been implicated in the development or prevention of FA. Vitamin D, in particular, has been implicated in dendritic cell and Treg cell tolerogenic activity [39–41], and may directly induce tolerogenic behavior in B cells [42]. Its potential role in FA is supported by geography – prevalence of allergic disease increases with distance from the equator [43–47] – and has been hypothesized as a potential mechanism involved in the association of fall (and to some degree, winter) births with higher FA incidence [48]. However, findings when evaluating serum vitamin D measurements in allergic and nonallergic patients have been inconsistent, with some studies finding a positive association between increased vitamin D concentrations and increased FAs in addition to those studies finding a negative association [49–51].

Other dietary and nutritional factors associated with FA include: dietary fats (omega-6 vs omega-3 long-chain polyunsaturated fatty acids, with the latter inconclusively positively associated with decreased FA incidence); antioxidant supplements such as vitamin C, E and β-carotene; vitamin A; zinc; and a Mediterranean diet [52].

Diagnostic tools for FA

The need for precise clinical tools is necessary to detect not only the presence of a possibly

life-threatening allergy, but to also predict the severity and prognosis of disease. The mechanisms of FA are not well understood and it is yet to be determined whether FA represents pathological immune deviance in allergic children or the absence of protective mechanisms normally found in a healthy child.

In one of few studies to date, Turcanu *et al.* analyzed the immune profile of peanut-allergic subjects, allergic subjects who had outgrown allergies and nonallergic subjects. Analysis of peanut-specific lymphocytes revealed the cytokine profile was polarized towards Th2 cells in peanut-allergic children, while nonallergic children and those that had outgrown allergies exhibited a Th1 response to stimulation [53]. All subjects were Th1-biased in response to nonallergic foods, suggesting an association between Th1 response and allergy resolution. In a subsequent study, Thottingal *et al.* argued against the idea of a protective Th1 bias in healthy individuals after finding insignificant differences in Th1 response between healthy and allergic subjects [54]. Taken together, these studies suggest allergen sensitivity does not innately alter the immune system, but instead determines immune response. Among children with milk allergy, 80% outgrow allergy by the age of 5 years [33], while only 20% of peanut-allergic patients outgrow their allergies [7]. Refined diagnostic tools are needed to assess predictive factors for spontaneous resolution versus allergy persistence, as well as for determining candidates for immunotherapy. There is still limited knowledge on the conditions that cause this shift from a healthy to allergic state, highlighting the need for increased studies profiling these differences.

Comprehensive guidelines have been developed to assist clinicians in differentiating between IgE-mediated FA and intolerance (adverse reactions that are not immune-mediated) [33,55]. The primary concern in diagnosing FA is patient safety, emphasizing caution to prevent false-negative diagnoses. Current diagnostic techniques emphasize the importance of clinical history, family history, presence of other allergic conditions and the timing of allergic symptoms following ingestion [55]. This history serves as a pretest assessment; if allergy is probable, diagnostic tests can be used for further evaluation. At present, skin prick testing (SPT) and measures of food-specific IgE (sIgE) are widely used in clinical settings [33,56,57]. SPT and sIgE are considered safe and can be used to predict the probability of a positive reaction to an oral food challenge (OFC).

SPT involves the application of food extracts to the skin accompanied with a slight puncture. Allergic patients present with a wheal on the skin when stimulated with an allergen. Wheal size is used to determine the likelihood of a positive reaction to OFC, with a positive predictive value (PPV) of >90% [55,57]. SPT can also be used to predict the likelihood of milk allergy resolution [58]. While SPT is safe, rapid and highly sensitive, it does not provide specific information regarding severity [55,59]. Extracts are often crude and unstandardized. Accuracy can be affected by factors like antihistamine use (false-negative) and pre-existing atopic dermatitis (false-positive) [55,59]. Diagnostic accuracy in SPT can be enhanced by focusing on single protein components, for example, casein in milk-allergic patients [60]. Multiple components, however, must be tested to ensure that allergy to any protein is not missed. Titrating allergen extracts in serial dilutions has also increased accuracy up to 99% [61]. Studies suggest a high PPV for SPT, however, it is important to note that this predictability varies from study to study given variations in age, allergen and the methods used in the food challenge. In addition, high PPV for SPT is generally associated with a large wheal size; many patients present with small-to-medium reactions, which often do not fall within the cut-off value described [62].

Mechanistically, levels of sIgE have been positively correlated with the production of antigen-specific Th2 cytokines [63]. In IgE-mediated FA, measurements of sIgE have been shown to correlate with the likelihood of a clinical reaction [7]. In patients aged 4–11 months, measurements of sIgE are more sensitive than SPT [64]. However, levels of sIgE do not always correlate with reaction severity or clinical threshold of tolerance [7,59,65]. Concordance between positive SPT and high sIgE in milk and egg allergies was found to be very low, indicating that these two diagnostic tools are not interchangeable, but work best in conjunction [66]. sIgE is measured in the serum via solid-phase ELISA using commercial technology such as ImmunoCAP® (Phadia AB, Uppsala, Sweden). A limitation of this technique is that results must be interpreted on an individual basis based on clinical presentation, since specific cut-off values for sIgE are hard to identify. In addition, these cut-off values are based on small study groups and vary from individual to individual [55,67]. While some guidelines are predictive of a 95% chance of reaction [57], it is important to note that in 10–25% of reactions, sIgE can be virtually undetectable [7].

As with SPT, sIgE measurements are being refined to look at specific epitopes using component resolved diagnostics (CRD) [7]. In CRD, a pure allergen is generated either from a natural

source or through recombinant expression of allergen-encoding DNA and used for subsequent testing. Measuring the sIgE to specific proteins, such as Ara h 2 for peanut, is much more precise (97% accurate; sIgE >0.35 kU_A/l) with a narrower cut-off than the use of the entire food (82% accurate; sIgE >15 kU_A/l) [56]. Specific epitopes are also useful in predicting the persistence and severity of a FA [56,68,69]. In milk allergy, the binding diversity of IgE has been linked to increased allergy severity [68]. sIgE to Ara h 1, Ara h 2 and Ara h 3 are indicative of severe and persistent peanut allergies, while Ara h 8 binding was associated with allergy in only 17% of patients [56]. In an additional study, monosensitization to Ara h 8 was found to indicate tolerance, suggesting that CRD could be useful in discriminating between allergic phenotypes [69]. Sensitization to Ara h 9 is linked to peanut allergy in the Mediterranean, suggesting that CRD can also be used to investigate regional differences [70]. For egg and milk allergies, ovomucoid-sIgE and casein-sIgE, respectively, are markers for persistent allergy [56]. CRD could also help clinicians identify patients who will have persistent allergies and advise them to permanently avoid the causative allergen, possibly preventing life-threatening anaphylaxis.

Protein microarrays can simultaneously measure IgE binding to a number of different components. The ImmunoCAP ISAC® system (Phadia AB) can measure sIgE for up to 112 allergens, requires 30 µl of plasma and takes less than 4 h. In this system, serum is added to a chip coated with immobilized allergen components. sIgE is measured based on luminescence [56,71]. Research is underway to develop automated microarray systems using photoimmobilized allergens [72]. While micorarrays may not enhance diagnostic capacity [73], this technique requires little sera, making it ideal for detecting sIgE in young children. Microarray technology allows for rapid measurements of many components to allow diagnoses to be made precisely, accounting for geographic location, individual sensitization and cross-reactivity [71]. This technology could be useful for identifying candidates for therapy and as a monitoring tool during treatment.

IgE is measured routinely in clinical laboratories; however, measurements of other antibodies such as IgG are generally limited to research settings. Similar to IgE, IgG is measured with ELISA and compared with a standard curve generated using purified human IgG. High levels of serum IgG have been reported in tolerant individuals, but were also found in allergic patients [74]. In addition, IgG4 levels may reflect past allergen exposure and, thus, are not indicative of tolerance level in patients undergoing oral immunotherapy (OIT) [65]. Binding of IgG4 epitopes to milk-specific proteins had no correlation with disease severity [68] and baseline levels of serum IgG4 were not predictive of allergy resolution [58]. In one study, casein-specific IgE/IgG4 ratio was used to accurately discriminate between tolerant patients and those reactive to baked milk, but was not effective in discerning desensitization to heat-inactivated protein (baked milk) from those who had fully outgrown allergy [65]. Measurements of IgG have not shown optimal predictive value, but routinely measuring IgG alongside IgE in allergy testing could provide further insight into the immune profile of allergic, desensitized and tolerant patients. Measurements of IgE/IgG4 ratios appear more promising than measurements of IgG4 alone. In a study of patients undergoing sublingual immunotherapy (SLIT) for peanut allergy, Kulis *et al.* found salivary peanut-specific IgA correlated with food challenge outcomes, although serum sIgA did not [75]. This study is the first to measure salivary IgA in patients undergoing SLIT with peanut protein, and suggests that salivary sIgA and serum IgA levels are correlated. Expanding studies on salivary levels of other antibody subclasses may present a minimally invasive technique in the study of FA.

One suggestion for improving diagnostic resolution of antibody quantifications would be to test functionality through *in vitro* tests such as the basophil activation test [65,76,77]. IgE binding to FcεRI receptors on the basophil results in activation, marked by increased CD63 and CD203c expression. Basophil studies are especially promising, given findings that basophil suppression is associated with desensitization in immunotherapy [78,79]. However, basophil activation tests are time- and resource-intensive, and the use of flow cytometry limits them to a laboratory setting [56]. This assay does hold promise for monitoring allergic patients on therapy [77], but must be standardized to allow for comparison between patients and different studies.

Despite new developments, OFC is still considered the 'gold standard' for confirming positive or negative diagnoses. Types of OFC include open, single-blind and double-blind. Blinding of patients and observers minimizes bias. The most meticulous strategy is the double-blind placebo-controlled food challenge (DBPCFC). In a DBPCFC, patients and observers are blinded and all materials are prepared by a third party. DBPCFC is especially useful for patients

with multiple allergens in order to differentiate between true allergies and food aversions. If a patient passes a DBPCFC (i.e., has no reaction), an open food challenge should be performed before reintroducing the food. An OFC is particularly useful when, based on SPT and sIgE testing, a positive reaction is highly unlikely and the challenge can be used to effectively convince patients and family that the food can be safely reintroduced. OFC is also useful to confirm if a patient has outgrown their allergies as is relatively common in allergies such as milk and egg [57,59].

While an OFC can accurately confirm a diagnosis, procedural variability exists and efforts have been made to standardize stopping criteria to maximize both safety and consistency [57,59]. Despite monitoring precautions based on clinical preassessment and extensive guidelines, there is always risk of adverse reaction and constant medical supervision is needed. Reactions can be latent, so patients must be monitored for several hours after the challenge. OFC can also cause anxiety in patients who have experienced life-threatening anaphylaxis following accidental ingestion. In addition, for patients with sensitivity to multiple allergens (~30% of the allergic population [26]), OFC for each food must be spaced out and can take days when testing cross-reactive foods [57,59].

Refined diagnostic techniques may minimize the need for OFC and maximize safety by helping predict reaction severity. Using sIgE and SPT measures coupled with CRD, the likelihood of reaction during OFC can be predicted with a PPV of up to 99%. We predict that the refinement of CRD and high-resolution epitope microarrays will allow for accurate, individualized results, allowing clinicians to confidently suggest exclusion of the antigenic food without the time, cost and anxiety associated with OFC.

Treatment of FA: current & future potential

There are several therapies currently under investigation for FA, some more promising than others. A summary of these therapies follows.

Nonfood-specific immunotherapy

Nonspecific immunotherapies do not target a specific allergen and instead target general immune mechanisms. Advantages of nonspecific immunotherapies include the potential ability to desensitize an individual to multiple allergens at a time, as many food-allergic individuals have more than one allergy [26].

One of the most promising nonspecific immunotherapies is a formulation of herbs used in traditional Chinese medicine, titled food allergy herbal formula (FAHF). Two different formulations have been studied. FAHF-2, the combination currently under study, is a simplification of FAHF-1, containing nine herbs instead of the 11 in FAHF-1. Both have been shown in mice to be effective in protecting against peanut-induced anaphylaxis in a mouse model of peanut allergy [80,81]. Promisingly, FAHF-2 was found to be effective in protecting against multiple allergic reactions in a mouse model with peanut, codfish and egg allergies [82]. Two Phase I trials for FAHF-2 have been conducted and found the formulation to be safe and tolerated; in the first, it was found that peripheral blood mononuclear cells treated with FAHF-2 *in vitro* produced decreased IL-5, and increased IFN-γ and IL-10 [83]. The second was an extended Phase I trial, lasting 6 months; FAHF-2 was safe and tolerated, and subjects demonstrated a decreased basophil response [84]. A Phase II trial to assess safety, efficacy and immunomodulatory effects is currently underway [201].

Another particularly promising therapy is anti-IgE therapy, specifically the use of the monoclonal anti-IgE antibody omalizumab. Omalizumab binds free IgE, therefore preventing degranulation of basophils and mast cells and attenuating Th2 cytokine production; it has also been found to reduce symptoms of IgE-mediated asthma [85]. A Phase II trial of the use of omalizumab in peanut-allergic patients has been performed. While the study was halted early due to safety concerns during the entry food challenge, primary end point analysis demonstrated an 80-fold increase in tolerated dose in omalizumab-treated patients versus placebo [86]. As omalizumab targets all IgE, it may also show some effectiveness in treating multiple allergies. Omalizumab also shows promise as an adjunctive therapy for other immunotherapies, as demonstrated by Nadeau *et al.* in their pilot study combining omalizumab therapy with milk OIT [87]; several trials building on this idea are currently underway [202–204].

Given the notable differences in gut flora between food-allergic and nonallergic individuals [24,25], the use of probiotics – "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" [205] – for treatment and/or prevention of FA is currently under study. While promising results have been shown in mice, especially in the administration of probiotic bacteria transfected with IL-10 or IL-12 [88,89], probiotic trials in humans have, overall, been disappointing [90].

There is, however, growing promise that the Human Microbiome Project [206], which seeks to deep-sequence 16S ribosomal subunits of bacterial RNA, may help in identifying targets for gut biota manipulation, leading to more targeted and promising therapies [2].

Other nonspecific therapies include: the use of parasites, specifically the helminth *Trichuris suis* ova, which has been found in mice to protect against IgE sensitization and anaphylaxis [91] and in humans to modulate ulcerative colitis [92] and Crohn's disease [93], and which is currently under investigation in a Phase I trial [207]; and the use of agonists of Toll-like receptor 9, stimulation of which induces systemic and mucosal Th1 responses, and which in mice have been found to protect against peanut-induced anaphylaxis, both during and post-sensitization [94]. Toll-like receptor 9 agonists may also show promise as adjunctive therapies [95].

Food-specific immunotherapy

Specific immunotherapies target specific allergens, and are particularly promising as monotherapies; multiallergen-specific therapies are less common, but are beginning to emerge.

SLIT involves the administration of micrograms or milligrams of food, which are held under the tongue for a period of time and then either spat out or swallowed [96]. SLIT is currently widely used in Europe to treat environmental allergies; while it is also used in this way in the USA, it is still considered experimental and lacks US FDA approval [97]. SLIT has been studied for use as immunotherapy for hazelnut [98], milk [99,48], peach [100] and peanut [101] allergies; multiple trials are ongoing [208–210]. Overall, SLIT has been found to increase the threshold of tolerated allergen, as well as allergen-specific IgG4, an indicator of tolerance. Various studies have also found increases in IL-10 [98] and salivary-specific IgA [101], as well as decreased skin reactivity [48,100] and IgE [101]; others have found increased [100] or unchanged IgE [98,99]. A major advantage of SLIT is its high safety profile; most reactions are limited to localized oropharyngeal symptoms [102]. The challenges of SLIT are a lack of dose standardization [97], relatively small maximum doses and potential for developing new sensitizations to related allergens [103]. SLIT may be especially useful in combination with other therapies that allow desensitization to higher amounts of allergen, such as OIT; In addition, given its effectiveness in environmental allergen treatment, it may prove useful for treatment of oral allergy syndrome (OAS).

OIT is currently under investigation and involves the oral administration of small doses of a causal allergen over time, with gradual dose escalation. Rapid desensitization can be achieved through heightened dose escalation and has been found to be enhanced with the addition of anti-IgE antibodies [85]. Despite some success in desensitizing many subjects with persistent FAs, OIT is associated with significant safety risks. Initial dosing must be performed in a setting monitored by health professionals, as most reactions occur during initial dose escalation and can require epinephrine administration to mediate anaphylactic reactions [104]. Epinephrine, if administered expediently, can be life-saving, yet recent studies highlight severe underuse in emergency room settings [105]. Thus, to ensure safety, monitoring should be coupled with patient education on treating anaphylaxis should a reaction occur outside the clinical setting. Even with extensive precautions, OIT is not suitable for all subjects, and many trials report adverse reactions during treatment. In a study on egg OIT [106], reactions accompanied 25% of all OIT doses given and nearly 13% of the OIT subject group stopped dosing due to allergic or anxiety reactions. Another study deemed cow's milk OIT "insufficiently safe" for 25% of participants based on their reaction severity [107]. Similar trends are seen in studies of peanut OIT with up to 18% of participants unable to cope with side effects [108–113]. Eosinophilic esophagitis, allergic inflammation of the esophagus, is a condition that has been linked to FA in children [114] and has been reported to occur during some OIT treatments [114–116].

Several meta-analyses on immunotherapy, as reviewed by Sampson, show that while OIT can be beneficial, it is not currently recommended for routine use [117–119]. Moreover, the response to OIT may not be uniform in all subjects. Keet *et al.* followed patients for an average of 4.5 years after ending milk OIT and found a majority of subjects limiting milk consumption due to adverse symptoms, with two reactions severe enough to require epinephrine use. One participant passed a 16-g challenge without symptoms, but became reactive again at follow-up [120]. Larger, randomized trials are necessary to investigate cost–effectiveness, safety and long-term efficacy.

Retrospective analyses are being performed on study cohorts to identify potential factors that could predict the probability of an adverse reaction. Risk factors for reaction in cow's milk OIT include high sIgE, SPT wheal diameter and

reaction severity during baseline OFC [107]; however, these parameters cannot be generalized for all allergens and OIT protocols. These data are based on individual studies and, as of yet, OIT has not been standardized, further complicating interstudy comparison. Improved specificity in diagnostic tools could allow clinicians to discern the likelihood and severity of a response during initial OIT dosing. These tools could also allow for the customization of the dosing regimen to improve both safety and efficacy.

Combining OIT with anti-IgE therapy has been suggested as a means of minimizing reactions during treatment. Prior studies have shown that monoclonal antibodies to IgE, such as omalizumab, can increase the threshold for reactivity to an allergen [121], but do not significantly minimize the rate of adverse reaction. Anti-IgE functions by neutralizing unbound IgE and preventing interaction with IgE receptors on basophils and mast cells that would elicit an allergic response [122]. In a study from Bedoret *et al.*, coupling milk OIT with omalizumab allowed for rapid dose escalation and resulted in nine out of ten subjects tolerating >8000 mg of milk in just 24 weeks [79]. In addition, all patients were able to complete 52 weeks of the study and continue with maintenance dosing to maintain desensitization. Subjects included in the study had high levels of milk-specific IgE, indicative of persistent allergy [79]. High levels of sIgE have been associated with a high likelihood of reaction [56], suggesting that adjunct anti-IgE therapy with OIT would be well suited for more sensitive patients.

The studies described illustrate that patients undergoing OIT can become desensitized to food allergens; the potential to achieve long-term tolerance is still under investigation. Desensitization is a state of unresponsiveness to antigen while continually taking OIT doses. 'Tolerance' is considered when the antigenic food is removed from the diet for a period of time and, when reintroduced, still does not elicit an allergic response. Longitudinal studies are needed to determine the ideal dosing scheme to not only desensitize, but to also possibly tolerize patients. In a study by Burks *et al.*, immune markers were studied that could differentiate patients who will achieve tolerance from those who will not. Increased levels of egg-specific IgG4 and small SPT wheal diameter were associated with passing an OFC, 4–6 weeks after discontinuing OIT [106]. Baseline characteristics may also be used to possibly determine the likelihood of developing tolerance. In studies by Jones *et al.* and Burks *et al.*, and others, patients with lower peanut-specific IgE and smaller SPT before starting OIT were more likely to tolerate OFC after discontinuing OIT for 1 month [108,123].

To date, OIT has not been shown to induce long-term tolerance, with studies extending for 2 years at most, and we should be careful before drawing any firm conclusions on its long-term effects [119]. Studying tolerant and nontolerant cohorts for extended time periods is necessary for exploring the possibility of inducing long-term tolerance.

The mechanisms involved in the development of desensitization and tolerance following OIT are still unclear, but appear to modulate the immune profile. Studies measuring immune parameters report changes in the number of Tregs [124]. Tregs are modulatory cells that play a crucial role in immune tolerance and help attenuate allergen-mediated responses through their suppressive function [125]. Increases in IgG4 production [95], decreases in proinflammatory Th2-type cytokines and suppressed basophil activation [126] have all been associated with OIT and could be enhanced by suppressive Treg cytokines. The development of Tregs appears to be dose-dependent; low dosing results in the development of antigen-specific Tregs, while high-dose OIT results in T-cell effector anergy, but no Treg development [79]. In FA, antigen presentation occurs at the intestinal level, so the migration of Tregs, as well as the development of tolerogenic dendritic cells, is also being explored as a possible mechanism of induced oral tolerance [127–131]. Mechanistic understanding of induced tolerance may help us determine if Treg development or T-cell effector anergy is necessary for sustained unresponsiveness. These findings could help determine if slow or rapid dose escalation is optimal for achieving tolerance. In addition, mechanistic studies could aid in the development of diagnostic tools to measure OIT progress.

The mechanisms of specific immunotherapies such as OIT and SLIT are thought to be similar [128], but variations in dosage may account for differences in the amount of antigen tolerated between therapies. Compared with OIT, SLIT is associated with localized, less severe systemic reactions. Having patients undergo SLIT followed-up by OIT could allow even hypersensitive patients to benefit from OIT. In a study performed by Wood's group, patients with milk allergy treated with SLIT followed by OIT allowed sensitive patients to undergo treatment and resulted in elevated levels of allergen

tolerance compared with SLIT alone [48]. The greatest limitation of OIT is the high rate of adverse reactions experienced during treatment. Combining other treatments with OIT could minimize adverse reactions and expand accessibility. Nonspecific therapies such as anti-IgE monoclonal antibodies and antihistamines are also promising. Before OIT is ready for clinical use, it must be standardized and made safer.

Subcutaneous immunotherapy (SCIT) is the most common immunotherapy for environmental allergies. However, clinical trials testing the use of SCIT to treat peanut allergy found it to be very dangerous, with high rates of systemic reactions, and its use has since been discontinued [132,133]. At the same time, SCIT for some environmental pollens does show promise as a potential therapy for OAS, an IgE-mediated FA found in patients with pollen or ragweed and characterized by oral allergic reactions to fruits, with similarity to said pollens (e.g., birch and apple and birch and hazelnut) [134]. Birch pollen SCIT has been found to decrease clinical sensitivity and skin reactivity to apple in some subjects, as well as to increase the tolerated quantity of apple and hazelnut in some OAS patients with birch allergy [135].

Another orally administered immunotherapy is the use of extensively heated egg and milk. Individuals who outgrow cow's milk or egg allergies tend to be allergic to conformational IgE epitopes instead of linear epitopes [136,137]; these epitopes may be disrupted by extensive heating, as occurs in baking, and so many of these children tolerate consumption of baked milk or egg products [138,139]. Regular ingestion of baked milk products has been associated with accelerated resolution of cow's milk allergy in children [140], suggesting complete avoidance is not the best path to take. This liberalization of the diet through the introduction of baked or heated allergens is associated not only with possible desensitization, but has also been shown to vastly improve patient quality of life by decreasing the anxiety associated with strict food avoidance [141]. One of the major advantages of this finding is that the use of baked milk or egg products in therapy is the ease with which it can be used in the home as well as the clinic, but it should be noted that, without proper monitoring and prior assessment of heat-treated protein tolerance, this can be extremely dangerous.

Epicutaneous immunotherapy (EPIT) involves the delivery of allergen via an epicutaneous patch containing solubilized allergen [142]. Peanut EPIT has been successful in mouse models [143,144]; to date, the only published human trial was a pilot study in children with cow's milk allergy [142]. The study found EPIT to be safe and well tolerated, with no significant change in IgE. At the same time, EPIT is relatively safe, presenting with mostly mild side effects and it is easy to administer [142]. Studies of peanut EPIT in humans are currently being conducted [211].

Intralymphatic immunotherapy, in which allergen is delivered directly to the lymph node via injection, is currently being assessed for treatment of human environmental allergies (specifically grass pollen [145–147] and cat allergen with an MHC class II-targeting modification [148]). It has shown great promise, having much greater efficacy than SCIT in far fewer doses, inducing stronger immune responses without polarizing T-cell responses [146,149,150]. Its use in FA therapy has yet to be assessed, although it has been found effective in a mouse model of ovalbumin allergy [146], and its efficacy in aeroallergen therapy suggests it is an avenue worth pursuing.

Other specific therapies modify the allergen proteins used to decrease the potential of reaction while still inducing tolerance.

Peptide immunotherapy involves the administration of a small fragment of the allergen that, while able to elicit a T-cell response, is too short to cross-link IgE on basophils and mast cells and thus does not induce anaphylaxis [151]. It has been shown to be effective for treating cat and bee venom allergies in humans [152], and egg and peanut allergies in mouse models [152,153], with mouse data suggesting induction of a Th1 shift and increased Treg markers [153]. The main issue with peptide immunotherapy is the development of the peptide fragments: while some progress has been made on peanut in humans and egg in mice [152,154–156], it is hard to produce a validated vaccine due to the complexity of creating a stable combination of multiple peptides in one vaccine [157]. At the same time, as animal trials and aeroallergen studies in humans progress, it seems FA peptide immunotherapy trials in human are not far off.

IgE-binding sites of allergens can be modified via site-directed mutagenesis or alteration of tertiary protein structure, a process that has been studied for quite some time in human environmental allergy immunotherapy [152,158]. Modified, hypoallergenic allergens that have been synthesized include peanut (Ara h 1,2,3) [159], milk (casein) [160,161], fish (parvalbumin) [162], peach (Pru p 1) [163] and apple (Mal d 1) [164]. Human trials have yet to be performed, but recombinant ovomucoid was found to decrease

anaphylaxis scores and histamine responses, while increasing IFN-γ, suggesting a Th1 shift [165,166]. Another modification being assessed is the addition of sugar moieties to the allergen to mimic pathogens and drive the Th1 response. Pretreatment with mannosylated bovine serum albumin (BSA) in BSA-sensitized mice was associated with significantly reduced anaphylaxis scores and lower BSA-specific IgE levels, via induction of IL-10 expression in dendritic cells via a C-type lectin receptor [167].

Bacterial adjuvants, such as heat-killed *Listeria monocytogenes* and heat-killed *Escherichia coli* [168], when delivered with allergens, can skew the immune system to a Th1 response, as has been demonstrated in dog and mouse models of peanut allergy [152]. Phase I clinical trials to study the safety of rectal delivery of heat-killed *E. coli* and a mixture of modified Ara h 1, 2 and 3 are currently underway [212].

Finally, gene therapy may provide new directions. Bacterial plasmid DNA can be used to deliver genes of allergens or of cytokines important to tolerance (e.g., TGF-β), both of which have been found to be effective in modulating allergic responses in mouse models [169,170], although this seems to have a strain-specific effect in mice [171], suggesting consideration of human genetic variability will be necessary. Targeting genes found to be associated with development of FA may also be a promising avenue for preventing FAs, especially as our knowledge of the human genome and ways to manipulate it increases. At the moment, however, such therapies remain far off.

Conclusion & future perspective

FA is a complex, multifactorial disease with increasing prevalence worldwide. Research into the mechanisms and risk factors underlying FA has elucidated some of the features of this disease and have suggested potential avenues for treatment, although much remains unknown. Further understanding of FA mechanisms will likely come from studies of genetics and epigenetic factors, as well as enhanced demographic studies; new guidelines regarding maternal diet will likely be generated on the basis of early exposure studies. Although there has been an increase in the report of FA, diagnostics are currently imprecise, and must be refined in order to determine disease severity and the possibility of developing spontaneous tolerance, as well as to better understand the global impact of FA. We hypothesize that the development of sophisticated, high-resolution tools will aid in the development of diagnostics that are minimally invasive, low risk and individualized to hold the potential to identify and monitor candidates for therapy, conditions that are not met by current practices and tools.

Several potential therapies, both specific and nonspecific, are under investigation; of these, the most researched, and potentially most promising, is OIT. At the same time, researchers agree OIT is not ready for clinical use, given the high rate of associated adverse events and the risk of resensitization. Future studies must examine long-term effects of OIT, and seek to standardize protocols and monitoring. Combining OIT with adjunctive therapies such as anti-IgE, SLIT and modified allergens may potentially increase the safety, efficacy and feasibility of this therapy for FA. Other therapies that will likely become more heavily researched are SLIT, EPIT, intralymphatic immunotherapy, peptide immunotherapy and the use of bacterial adjuvants. At this time, however, OIT – and, indeed, all immunotherapies for FA – are highly experimental, and much more research is needed before any of these can enter the clinic. We cannot overstate the importance of studying both the mechanisms and patient outcomes in the assessment of potential therapies.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Food allergy is a complex disease that is increasing in prevalence, but the causes and risk factors remain unclear.
- Diagnostic tools for assessing food allergy are imprecise, and must be improved to enhance safety and test resolution.
- No therapy currently exists for food allergy; several specific and nonspecific immunotherapies for food allergy treatment are currently under different stages of investigation.
- Oral immunotherapy is a particularly promising intervention, but at present is experimental and requires extensive study to enhance safety.
- Combinations of therapies may provide the best avenue for the management and treatment of food allergy.

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