



Food allergy: management, diagnosis and treatment strategies

Food allergy is an increasing problem in western countries, with strict avoidance being the only available reliable treatment. However, accidental ingestion can occur and anaphylactic reactions still happen. In recent years, many efforts have been made to better understand the humoral and cellular mechanisms involved in food allergy, and to improve the strategies for diagnosis and treatment. This review focuses on IgE-mediated food hypersensitivity and provides an overview of the diagnostic strategies and treatment advances. Specific immunotherapy, including different routes of administration and allergen sources, such as natural, recombinant and T-cell epitopes, are analyzed in detail. Other treatments such as anti-IgE monoclonal antibody therapy, adjuvant therapy and Chinese herbs will also be described.

KEYWORDS: dendritic cell • desensitization • food allergy • IgE • immunotherapy • nonspecific immunotherapy • specific immunotherapy • tolerance • Treg

Food allergy (FA), the prevalence of which is increasing in industrialized countries, results from Th2-polarized immune responses to innocuous environmental proteins – so-called allergens [1]. Allergenicity (i.e., the capacity to induce the production of specific IgE [sIgE] antibodies) is restricted to a minute fraction of all proteins to which humans are exposed. More than 170 food proteins have been described as allergens and categorized into different families, although just a few of them, such as nuts, legumes, fruits, eggs, milk and seafood, are responsible for 90% of FA cases [2,3]. Although different immunological mechanisms, such as IgE-mediated, cellular-mediated or mixed IgE cellular-mediated, are associated with FA, in this review we will focus on the most frequent and best-studied, IgE-mediated reactions. The diagnosis is complex and in many cases the blind administration of the suspected food is the only available method, even though this procedure is not free of risk.

The current management of FA consists of strict avoidance of culprit foods, a proper counseling of the patients to recognize the early symptoms of allergic reactions and training to self-administer epinephrine [4]. Nevertheless, patients are not exempt from risk because accidental ingestion can occur and the presence of common allergenic components in different products may give rise to an anaphylactic reaction. Accordingly, new treatments are necessary in order to reduce or prevent the risk of suffering unwanted reactions, which would, in turn, improve the quality of life for these patients [5].

For many years, in allergic diseases such as allergic rhinitis, asthma, venom hypersensitivity and more recently in FA, allergen-specific immunotherapy (SIT) has been applied, with the main goal of inducing long-term desensitization or tolerance. SIT has been shown to reduce the symptoms and the symptomatic medication required. Moreover, SIT is able to induce long-term clinical effects and it is also able to prevent the onset of further allergies [6,7]. SIT is the only treatment able to modulate the immunological response and therefore the course of an allergic disease. Although promising results have been obtained, we are still far from understanding the complex mechanisms leading to the successful results in SIT. Some of these mechanisms will be analyzed in detail in this review.

Prevalence

Although the real prevalence of FA is still not well known, some data suggest it is increasing, at least in western countries [8–10]. Obtaining comparable prevalence data is difficult due to several factors, including the nonstandardized food allergens employed, the diagnostic criteria used and the characteristics of the study population (age, sensitization pattern). In 2007, the EuroPrevall consortium (EU-funded project on FA) undertook a meta-analysis of FA publications, including only those studies with clear FA to milk, egg, peanut, fish and seafood in children and adults. This study showed the prevalence of FA to be 1–5% of the total population in the European community [11,12]. Similar results were found in other studies based on a US population

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where 4.2% of children and 1.3% of adults were diagnosed as allergic [13]. Simultaneously, other studies have shown frequencies of FA of approximately 6% in children and 4% in adults [14,15]. Recently, a study including more than 2800 Australian children diagnosed by skin prick test (SPT) to one or more food allergens showed an increased prevalence, with more than 10% of patients with proven IgE-mediated FA to one of the common allergenic foods [16]. However, the real percentage could be underestimated since food allergens in vegetables and fruits were not included. This is important because while animal proteins are allergens frequently associated with FA in children, vegetables and fruits are the allergens more frequently involved in adults, especially in certain geographical areas, such as Mediterranean countries [17]. In addition, although reactions to vegetables and fruits frequently involve oral allergy syndrome (OAS), systemic symptoms nevertheless occur. TABLE 1 shows the most frequent allergens involved in children and adults with FA.

Immunological mechanisms involved

Although any food protein may theoretically become an allergen, only proteins belonging to a few families have so far been characterized as food allergens [3]. Research has focused on defining the properties that lead proteins to become an allergen, demonstrating that just a few features such as stability to heat and enzymatic processes, a size of 10–70 kDa and water-solubility, are the most important [10].

Table 1. Main allergens and reported percentages in food allergy in children (<16 years) and adults (>16 years).

Food	US children (%)	US adults (%)	European children (%)	European adults (%)
Milk	2.5	0.3	0.5–2	0.5
Eggs	1.5	0.2	0.5–2.5	0.5
Peanuts	1.4	0.6	0–1.5	2.5
Tree nuts	1.1	0.5	0–7.3	NR
Wheat	0.4	0.3	0.5	>3
Soy	0.4	0.3	0–0.7	0.2–2.9
Fish	0.1	0.4	0–0.5	0.5
Shellfish	0.1	2	0–0.5	1.4
Sesame	0.1	0.1	0.1–0.9	<1
Fruits/vegetables	NR	0.1–4.3 [†]	0.1–4.3, <0.5	0.4–3.5

[†]Estimated/ranged percentage.

NR: Not reported.

Data taken from [10,12,17].

Over the last few years, numerous food allergens from different sources including animals [18], pollens [19] and plants [20] have been described. The biochemical analysis of these molecules has shown that some of them are widely distributed among different food families. They share highly conserved linear sequence regions or similar 3D structures, and are therefore considered as pan-allergens [3,21,22]. Panallergens such as profilins [23,24], polcalcins [25,26], pathogenesis-related proteins (including nonspecific LTPs) [27], parvalbumins [28], seed storage proteins [29] or tropomyosins [22], are the greatest source of IgE cross-reactivity among patients, thus representing a major problem in skin testing and *in vitro* diagnosis (TABLE 2).

The oral and gut mucosa are constantly in contact with antigens from different sources such as bacteria and dietary proteins, and, under normal conditions, tolerance develops. Specific suppression of humoral (B cells) and cellular (T cells) responses, and nonspecific conditions (pH, mucous production, gut epithelium barrier and enzymatic activity) all contribute to decreasing the allergenicity of antigens and to maintaining the tolerogenic environment [30,31].

Focusing on the immunological response, the role of different immune cells such as dendritic cells (DCs) or Tregs is well known in the induction of oral tolerance in humans [32–35]. DCs are professional APCs situated in all tissues, including the GI tract. DCs are continuously processing antigen molecules and presenting them to naive T cells localized in secondary lymphoid nodes associated with the gut, mainly Peyer's patches and mesenteric lymphoid nodes, and they play a pivotal role between tolerance and active immunity, depending on the state of maturity and the cytokine microenvironment in which this takes place [36]. The role of DC maturation in food allergen presentation has been demonstrated *in vitro* for Pru p 3, the major allergen of peach belonging to the LTP family, by increasing the specific T-cell proliferation in peach allergic patients [37]. Furthermore, DCs with tolerogenic/regulatory functions have been reported since they are able to induce Treg differentiation. It is well established that DCs are able to maintain a tolerant environment in the oral epithelium, where they are exposed to a large variety of allergens [38]. In this sense, the capacity of plasmacytoid DCs to generate functional FoxP3⁺ T cells has recently been shown [39]. Thus, DCs have an important role in oral tolerance and the mechanism involved in SIT.

Treg suppression of effector responses [40–42] can be mediated by the production of

anti-inflammatory cytokines such as IL-10 and/or TGF- β [40,43,44]. The regulatory function of IL-10 is based on its capacity to induce the differentiation of IL-10-secreting Tregs, to block the production of inflammatory cytokines by T cells and to suppress the antigen presenting function by decreasing the expression of MHC class II and costimulatory molecules [45–49]. TGF- β , however, is a bifunctional cytokine that can exert either proinflammatory or anti-inflammatory functions [50], inhibiting the proliferation and effector functions of T cells and the activation of macrophages, stimulating the switching to IgA in mucosal tissues [51] and contributing to the differentiation of CD4⁺CD25⁺ Tregs [52]. Other cells, such as B cells [53–55], CD8 T cells [56,57] or NK cells [58], have also been shown to have a role in the tolerogenic response under certain conditions. In this sense, it has recently been reported in bee venom allergy that inducible IL-10-producing B cells were able to suppress antigen-specific T-cell activation, in association with a shift toward IgG4 production [59]. FA may arise when the mechanisms designed for maintenance of oral tolerance fail. As in any allergic response, this involves two different phases (FIGURE 1): initial sensitization occurs once DCs recognize food allergens, migrate to secondary lymphoid nodes and present the allergen on MHC-II molecules to naive T cells. When this happens with the expression of costimulatory molecules and Th2 cytokines, a switching to an IgE isotype occurs in B cells [48]. This sIgE is bound to the high-affinity receptors (Fc ϵ RI) on basophils and mast cells; effector phase occurs when the subject is re-exposed to the specific allergen that crosslinks with several IgE bound to cell surfaces. This activates cells and triggers a degranulation process with release of different molecules such as vasoactive amines (histamine), lipid mediators such as prostaglandins or leukotrienes (LTc4, LTe4 and LTd4), or PAF and ECF-A. In addition, there is an induction of chemokines and cytokines responsible for the recruitment of other effector cells (eosinophils or effector Th2 cells), leading to inflammation and damage in the tissues [43,60–62].

Diagnosis

The diagnosis of FA is based on a clinical history and different *in vivo* and *in vitro* tests [1]. The former is the first approach to obtain information about the presumed source of the culprit allergen, the type of reaction according to the time elapsed between the intake and the appearance of symptoms, and to elucidate whether

Table 2. Panallergen families: size, sequence conservation (in closest species) and main sources of the main panallergens reported.

Most common panallergen families		Size (kDa)	Sequence identity (%)	Sources
Plant profilins		12–15	≥75	Pollen, food, latex
nsLTPs		9–10	30–80	Pollen, food, latex
Polcalcins		9	≥67	Pollen
Tropomyosin		65–70	≥50	Animals
β -parvalbumin 2S		12	≥53	Fish
Seed storage proteins	2S albumin	9.5–20	18–40	Kernels, nuts, seeds
	7S vicilin	30–80	NR	
	11S legumin	40–60	NR	

NR: Not reported; nsLTP: Nonspecific LTP.

other cofactors such as alcohol consumption or exercise could be involved [63]. IgE-mediated reactions are characterized by an early onset of symptoms, normally within 2 h after food intake and include clinical symptoms with different degrees of severity, such as mild (OAS), moderate (urticaria) and severe (GI disorders or anaphylaxis) [64]. The OAS is characterized by the presence of itching, stinging pain and vascular edema of the lips, tongue, palate and pharynx that can be accompanied by itching on the ear and a feeling of tightness of the throat [65–67]; urticaria, with or without angioedema, which usually appears as transient rapidly evolving pruriginous wheals at different sites of the body [64,68]; and anaphylaxis, the most severe reaction, which presents with systemic symptoms such as dyspnea, vomiting, abdominal pain, diarrhea and even hypotension that may cause death [69–72].

Among *in vivo* tests, the oral food challenge (OFC) is considered the gold standard to identify the specific food and to confirm the allergy, but it is frequently related with the risk of inducing an anaphylactic reaction [73–75]. Thus alternative *in vivo* tests, such as the SPT, or *in vitro* tests, such as fluorescent-enzyme immunoassays (FEIA-CAP) or radioallergosorbent tests (RAST) are also employed. These tests are based on the detection of sIgE in skin or in blood, although neither are truly predictive of allergy and, rather, they are considered as indicators of sensitization [76,77]. Moreover, these tests are unable to differentiate either cross-reactivity or the severity of the reaction [78]. Consequently, considering that skin test and *in vitro* test sensitivity is not optimal, negative results cannot be considered for allergy exclusion, since patients with undetectable levels of sIgE, who

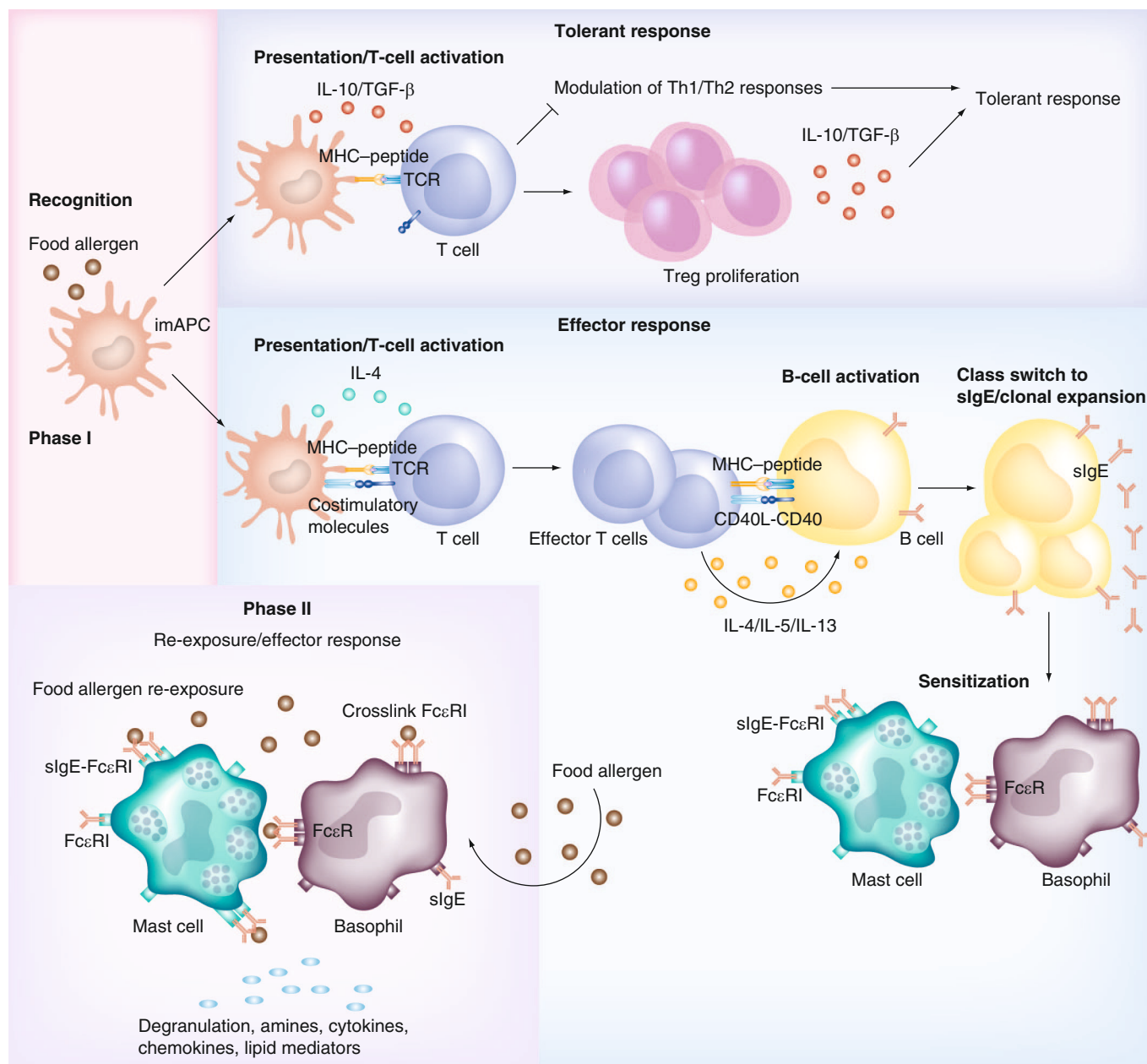


Figure 1. Mechanisms proposed in the development of tolerant and effector responses to food allergens. Phase I: Sensitization: once dendritic cells recognize food allergens, they are presented on MHC-II molecules to naive T cells. In tolerant responses, the absence of costimulatory molecules expressed on APCs and the production of IL-10 and TGF- β induce expansion of specific Tregs and a decrease in Th2 cell frequency, leading to a state of tolerance. However, DCs express costimulatory molecules and Th2 cytokines, inducing the switching to an IgE isotype in B cells and Th2 differentiation in T cells. sIgE binds to high-affinity receptors (Fc ϵ R1) on the surface of basophils and mast cells for a faster response in subsequent encounters. Phase II: Effector phase: re-exposure to the specific allergen, crosslinked IgE binds to cell surfaces and activates effector cells, triggering a degranulation process, with release of different molecules such as histamine, PG-D, LTC₄, LTE₄, LTD₄, PAF and ECF-A, and chemokines and cytokines, leading to inflammation and tissue damage. imAPC: Immature APC; sIgE: Specific IgE; TCR: T-cell receptor.

are skin test-negative and with normal tryptase levels can still elicit an anaphylactic reaction after OFC [79]. In the last decade, the inclusion of relevant food allergens has improved the predictive value of the tests. The determination of sIgE levels for Ara h 2 improved the diagnostic value to 60% when compared with whole

peanut in IgE determination or SPT (26 and 57%, respectively) [80].

Another *ex vivo* method, the basophil activation test (BAT), has been considered [81,82]. The role of the BAT in FA has been analyzed in patients allergic to wheat [83], egg and peanut [76,84], hazelnut [85], sesame seeds [79], cow's

milk [86,87] and mare's milk [88], and has demonstrated its value as a diagnostic tool. BAT is based on the determination of activated basophils after specific-allergen stimulation by flow cytometry techniques using monoclonal antibodies against IgE, CCR3, CD203c or CD123/HLADR for basophil characterization and CD63 or CD203c to determine their activation [89].

The heterogeneity of the allergens employed in *in vivo* and *in vitro* tests is important, being critical to control different factors such as the origin of food or its preparation (heated or not) [75,86] and the origin of manufactured products [90]. Nonetheless, to avoid the problem of the composition of the extracts, new approaches involving pure natural extracts and molecular techniques have been applied in allergy and termed component-resolved diagnosis (CRD). This test includes pure antigenic molecules from relevant allergens that are obtained from different sources, either produced by recombinant expression of allergen-encoding cDNAs or by purification from natural allergens. Several studies have evaluated the usefulness of CRD tests in comparison with other IgE-based tests, as well as their capacity to discriminate between allergic and sensitized subjects. It has been shown that CRD analysis is able to discriminate between patients with allergy to peanut (mainly positive to Ara h 2) and patients who, although sensitized, are still not allergic [91]. Similar applications have been obtained in other food allergies such as kiwi [92], milk and egg [93,94].

The use of epitopes instead of allergen extracts may define the profile of sensitization of the patients more accurately and therefore the diagnosis. The inclusion of many sequential epitopes from several food allergens in microarray techniques would make the diagnosis in FA more feasible [95,96]. This approach has been studied for lentil [97], egg [98], peanut [99], shrimp [100] and milk [101,102] epitopes, showing a correlation between sIgE or IgG antibodies and the clinical sensitivity; this has also been proposed as a tool for evaluating the effect of immunotherapy (IT) [103]. Although CRD and epitope use are promising, further studies must be done to establish their values in *in vitro* tests for evaluating IgE-mediated allergies and their correlation with *in vivo* tests such as OFC.

Treatment

Although avoidance has generally been the main treatment, this is not optimal. Thus, new approaches directed at inducing tolerance have recently been applied.

■ Avoidance & medical treatment

Traditionally, the treatment of FA consists of strict avoidance of the known or suspected food(s) that cause the reactions, in combination with appropriate counseling of patients, providing information to enable recognition of the early symptoms of the reactions and instructions to self-administer injectable treatments such as epinephrine. The identification and the recognition of culprit foods could be an alternative for these subjects. However, despite the many efforts by the various health administrations to clarify and include a clear and easy identification of all ingredients that must appear on the labeling of foodstuffs [201], the complete avoidance of some of the most common foods involved in allergy such as milk, eggs or fish is difficult, and accidental ingestion can result in anaphylactic reactions [104]. This is more difficult for those patients with multiple food allergies and in those with allergy to vegetable food that can have cross-reactivity not only between vegetables but also with pollen. Moreover, the withdrawal of some of these foods from the diet could lead to nutrition problems, particularly in the pediatric population [105,106].

■ Allergen-specific food IT

Allergen-specific food IT has been reported to induce an immunomodulation, as well as a desensitization state in patients through the daily exposure to increasing doses of food allergens [107–109]. This produces a transient state that allows patients to ingest small amounts of the food allergen, reducing or avoiding the life-threatening risk associated with accidental ingestion and improving transiently their quality of life. The goal of these therapies is the achievement of clinical tolerance, inducing permanent immunological changes, without the requirement for daily exposure. Nevertheless, the onset of tolerance has still not been established and although some reports suggest achievement of tolerance, it still remains elusive.

Nowadays, there are different routes of administration, including subcutaneous IT (SCIT), oral IT (OIT), sublingual IT (SLIT) and epicutaneous IT (EPIT), which are under evaluation in different clinical trials.

Subcutaneous IT

SCIT has been used for over a hundred years in different allergic diseases such as asthma, allergic rhinitis/rhinoconjunctivitis and hymenoptera hypersensitivity [110,111]. In FA, some trials have been undertaken with peanut-allergic patients. These studies demonstrated, by using aqueous

peanut extract, their benefit through the reduction of the symptom score and skin test reactivity in almost all the patients as compared with an untreated group [112,113]. However, the rate of significant adverse events, such as anaphylactic reactions, together with the high cost of the time-consuming treatment, requiring the administration of up to 80 injections over 4–5 years, limits its acceptance among patients and hinders its application [114]. Therefore, safer and less traumatic alternatives such as noninvasive routes of administration are currently under development.

Oral-specific IT

OIT as a route of IT administration is promising due to its safety compared with SCIT and the successful results. OIT consists of the regular and gradual oral administration of escalating doses of food allergens during the build-up phase. Once patients have achieved the maintenance dose, they continue the treatment at home with regular daily ingestion for years, with doses that allow them to accept the accidental ingestion of the specific food allergen without a reaction. What is achieved is a desensitization state, dependent on the regular administration of the treatment. Most of the studies published during the last century have shown desensitization in a considerable percentage of cases (50–75%) receiving OIT [115]. Recent studies show that some patients are able to incorporate the allergen into their normal diet after finishing OIT [116], although whether this indicates tolerance is difficult to determine, particularly considering that the studies have mostly focused on pediatric populations, where spontaneous resolution of the allergy can occur with some allergens such as milk or egg, which thus raises the question of whether these patients improved because of the OIT or as a result of the normal course of the allergy. Furthermore, follow-up studies for periods longer than 5 years are needed to confirm definitive tolerance.

Multiple studies involving milk, peanut and egg have been published, indicating that patients were able to increase the amount of allergen ingested [107–109,117–119]. A double-blind placebo-controlled study was carried out in children with milk allergy [118,120] and as a result they were able to tolerate higher amounts of milk, and immunological changes, such as increased IgG4 levels, were detected. In a double-blind, randomized, placebo-controlled study with 55 egg-allergic children, 11 of 40 patients in the OIT arm passed the OFC after 24 months of treatment and ten of them consumed egg *ad libitum*

without any reactions after 30 months [116]. Similar results were obtained in peanut allergy. Clark *et al.* reported in a short series of four patients that children allergic to peanut were able to increase peanut ingestion up to 50-fold after OIT and with all subjects tolerating IT up dosing to 800 mg protein without an epinephrine requirement [121]. Jones *et al.* also reported in a series of 29 patients that OFC carried out at different times during the maintenance phase showed that 93% of patients reached the total peanut dose of 3.9 g without severe symptoms, indicative of desensitization. Other immunological changes such as decreased basophil reactivity or SPT were also observed after 4 and 6 months of treatment, respectively, as well as a late decrease in sIgE level and an increase in specific IgG and IgG4 after 3 months of OIT [107]. However, it is important to underline that adverse reactions during OIT are common, both major transient and mild-severe reactions, and epinephrine administration may be required in a few cases, as has been established from Cochrane meta-analyses over recent years [107,120,122,123].

Sublingual-specific IT

This method consists of placing drops of the allergen extract under the tongue for a few minutes and then either swallowing it or spitting it out. The main advantage of this modality is the direct absorption of the allergen and quick access into the bloodstream. SLIT began to be applied as an alternative route of IT administration during the 1980s, being highly safe [124]. In 2005, Enrique *et al.* evaluated, in a placebo-controlled study, the effectiveness and safety of SLIT treatment in patients allergic to hazelnut. There was an improvement in the active group of the tolerated dose from 2.29 to 4.56 g, with no differences in the placebo group after 3 months of treatment, although no changes in the immunological parameters were found [125]. More recent studies with peach have also shown a reduction in reactivity in skin testing after SLIT treatment, with no changes in IgE levels after 6 months of SLIT with Pru p 3 [126].

Regarding peanut allergy, patients receiving SLIT with peanut extract during 1 year reached a dose of proteins 20-times higher in the oral challenge test than the placebo group. The outcome was accompanied by immunological changes such as reductions in IL-5 and IL-13 production, and IgE reactivity, or increased Treg and IgG4 production [127]. New evidence has shown a role of sIgA levels in SLIT. Kulis *et al.* have recently reported that changes in salivary peanut

sIgA levels strongly correlated with oral challenge outcomes [128]. Recently Fleischer *et al.* demonstrated, also in peanut allergy, that SLIT, after 44 weeks of treatment, was able to induce a modest desensitization, where 70% of the peanut-treated patients were able to pass an OFC with a dose of 5 g or tenfold more peanut powder than the baseline OFC (2 g), as compared with a placebo group [129].

Finally, the safety of SLIT has been shown despite the high percentage of adverse reactions, which include mainly transient or mild reactions, with no requirement for epinephrine treatment. SLIT therefore appears to be a promising treatment for FA, although more placebo-controlled trials are necessary in order to evaluate more accurately the efficacy, confirm the safety and determine whether it is capable of inducing permanent tolerance, which is the main goal of IT.

Epicutaneous-specific IT

The skin is an alternative route for IT, since it has a high frequency of APCs (mainly Langerhans cells) and its poor vascularization makes it interesting to avoid systemic reactions. EPIT consists of the repeated application of allergens to the intact skin [130,131]. Today adhesive tape stripping is the most frequently used method and its efficacy has been demonstrated with peanut allergy in an animal model. One of the main problems associated with using the skin as the route of allergen administration is the corneum layer, due to its low permeability. Attempts to resolve this have been made with the use of skin scratching with needles [130], or, more recently, a system in which high permeability is reached by hydration of the skin allowing delivery of the allergen into the stratum corneum and rapid uptake by DCs [132,133]. This method has recently been tested in humans in a double-blind, placebo-controlled study where a group of children suffering cow's milk allergy was evaluated to study the safety and acceptability of this technique. The authors showed an increase in the cumulative tolerated dose of milk after 90 days of treatment, with no systemic reactions detected. However, this study failed to demonstrate a statistically significant improvement, probably because a longer period of treatment is needed [134]. These data indicate that EPIT appears to be an alternative for IT that could be safer and more feasible than SCIT, although more studies have to be developed to confirm these results.

Modified recombinant protein vaccine

In order to reduce the adverse side effects of IT when using the whole allergenic protein, new approaches are being developed. The advances achieved in molecular techniques during the last decades have led to the identification of the main T and B epitopes of many allergens, such as Pru p 3 [135,136]. This understanding has helped the development of new hypoallergenic variants of many allergens, raising the possibility of using them in vaccines, which is an interesting conceptual approach to improve the safety and efficacy of IT. The site-directed modification of key amino acids of the allergens to alter their IgE epitopes decreases the activation of basophils and mast cells after recognition without affecting their ability to induce a proliferative T-cell response. This ability has been demonstrated mainly in murine models of peanut allergy. Moreover, in serum from allergic patients, mutated epitopes for Ara h1, h2 and h3 demonstrated a reduced capacity of peanut sIgE binding when compared with the native allergens. Moreover, it is important to mention that the modified allergens retained their ability to stimulate T-cell proliferation [137,138]. Li *et al.* also applied these modified peanut allergens in combination with bacterial adjuvants such as heat-killed *Escherichia coli* [139] or *Listeria monocytogenes* [140]. The effects of the daily administration for 3 weeks of heat-killed *E. coli* producing engineered Ara h1, 2, and 3 were evaluated by challenge at week 2, 6 and 10. Results showed that the treated mice exhibited reduced symptom scores compared with the untreated group and reduced levels of IgE, histamine and Th2 cytokines such as IL-4, IL-5, IL-10 and IL-13 after 10 weeks. These data, together with an increase in TGF- β and IFN- γ production by splenocytes, could reveal an immune deviation from a Th2 response to a Th1/Treg profile. A human Phase I trial with the recombinant modified peanut proteins Ara h1, 2 and 3 (EMP-123) finished in 2011 [202], although no results have yet been published. A similar strategy has been used with other allergens such as Mal d 1 (apple allergen), where a five-point modified Mal d 1 protein showed a decreased IgE-binding capacity in 10 out of 14 patient sera [141].

T-cell epitope IT

IT with peptides containing T-cell epitopes is also under research because they reduce basophil and mast cell stimulation, maintaining their ability to activate specific T cells. In FA, Prickett *et al.* recently reported a proof-of-concept study,

using peptides containing the sequences of the five dominant CD4⁺ T-cell epitopes of Ara h 2, showing that they are recognized by peripheral blood mononuclear cells and induce production of Th2 cytokines (IL-4, IL-5), but are not recognized by peanut-specific serum IgE from peanut-allergic patients [142]. Another study in a mouse model of egg allergy, using oral administration of single and multiple peptides containing the major T-cell epitope of egg allergen (ovomucoid), showed a similar deviation of the immune response [143]. This report showed that mice treated with the peptides had a reduction in clinical symptom score, histamine concentration (mainly when single peptide was administered), IgE reactivity and IL-4 production, together with an increase in the percentage of Treg cells and IL-10 production. It is important to note that these T-cell epitopes may have a lower capacity compared with native extracts to stimulate the immune system. In this sense, the use of adjuvants, such as agonists of Toll-like receptors, may be an alternative to induce the full response, increasing the efficacy of the treatment, since adjuvants are able to modify the immune response. An animal model has shown that the combination of CpG motifs (agonist of TLR9) with Amb a 1, the major allergen of ragweed pollen, is able to induce Th1-skewed immunomodulation [144]. In humans, clinical Phase I trials have been initiated in ragweed and house dust mite allergies, disclosing a good safety profile in short-term SIT [145–147]. Different clinical Phase II trials have also shown an induction of immunological changes and clinical efficacy from the increase in the Th1 cytokine profile and diminished eosinophilia [148] or increased production of Amb a 1 sIgG antibody levels [149]. Therefore, the use of adjuvants in combination with IT treatments may be a tool to improve IT.

■ Nonspecific IT treatments

These therapies attempt to alter the global immune response, not just against one specific food allergen. In fact, there is increasing interest in the role of new products based on Chinese herbal formulas and humanized anti-IgE treatments.

Herb formulas, containing a mixture of 14 different herbs, have been used in Chinese medicine for centuries and considered as a potential treatment for diseases such as asthma [150,151] or atopic dermatitis [152]. In FA, initial tests have been developed in a mouse model of peanut allergy, using an 11-herb formula (FAHF-1), demonstrating in treated mice reduced mast

cell degranulation, reduced Th2 cytokine levels, decreased peanut-specific serum IgE levels and a reduced *in vitro* lymphocyte proliferation, with a protective effect against peanut-induced anaphylaxis and no systemic adverse reactions or toxic effects [153]. Given the protective effect shown and in an attempt to make the formula safer and simpler, the same group reduced the number of herbs and developed a nine-herb formula (FAHF-2), demonstrating, in the same mouse model, that this new formula can also induce protection, with similar immunological changes to those described for FAHF-1, with an upregulation of IFN- γ produced by CD8⁺ T cells [154,155]. Recently, a mouse model of multiple allergies (peanut-, codfish- and egg-sensitized mice) showed that treated mice had no anaphylactic symptoms after challenge with each allergen and this was accompanied by the same immunological changes as those described above [156]. Clinical trials with FAHF-2 have been approved in the USA [157] and a Phase I study was developed in 19 randomized, double-blinded, placebo-controlled, dose escalation patients with different FA. Of these, only one patient suffered an allergic reaction but it was not attributed to the herbal formula due to the high atopic status of the patient and the negative SPT to all the herbal components. These results showed the FAHF-2 formula to be a safe and tolerable treatment for FA patients and a Phase II study is currently ongoing.

Recombinant humanized monoclonal anti-IgE has also been used as IT, with the idea that the reduction in IgE levels or Fc ϵ RI expression would reduce the IgE-mediated responses in food allergen exposure. The first trials were developed in 2003, when Leung *et al.* conducted a double-blind randomized study involving 84 peanut allergic patients. TNX-901, a humanized IgG1 monoclonal antibody against IgE, was administered at three different doses (150, 300 and 450 mg) in three groups of patients [158]. Only the higher dose induced a significant improvement in OFC performed 2–4 weeks after the last dose. In 2011, a Phase II clinical trial using the humanized monoclonal antibody omalizumab was undertaken in a group of 150 peanut-allergic patients. The results, based on an OFC performed 24 weeks post-therapy, showed that treated patients tolerated a higher amount of peanut as compared with baseline and with a placebo group [159]. Omalizumab has also been considered as a complement to OIT, for example in rapid oral milk desensitization treatment, showing a good safety profile since the

percentage of reactions after 2199 doses administered was 1.6% (32 events), with only 0.1% being severe [160].

Future perspective

FA is an increasing health problem in developed countries, affecting both children and adults, and impacting greatly on their quality of life. Nowadays, IT is the only etiological treatment in allergic diseases. Different IT approaches are under evaluation for each route of administration: SCIT, SLIT, OIT and EPIT. Most studies carried out have included well-characterized patients using both SPT and OFC, and with a double-blind placebo-controlled format, thus providing strength to the study. However, some limitations nevertheless arise with these studies, such as the reduced number of patients included, the follow-up period and the use of low concentrations and/or no well-characterized allergen. As an increase in allergen concentrations could enhance the risk of suffering an allergic response since whole proteins are included, many efforts have been made to improve the safety and efficacy of specific IT. The immunological mechanisms underlying IT are now better understood and it is well known that IT is able to readdress the immune response toward a Th1/Treg response instead of Th2 profiles, with a key role of different components of the immune system, such as cells with regulatory functions (Tregs, regulatory B cells, regulatory DCs and others), APCs (DCs) or messengers such as IL-10 or TGF- β , with

demonstrated involvement in desensitization or tolerance responses.

Although SLIT and OIT appear promising routes, they still require improvement in efficacy and safety. While SLIT has been shown to be safer, OIT seems to have higher efficacy. In order to increase the efficacy of SLIT, more concentrated allergen extracts should be developed. To increase the safety of OIT, however, combined approaches with omalizumab or SLIT [109] in the build-up phase have been proposed. Molecular advances will allow the development of a new generation of hypoallergens and vaccines based on modified proteins and T-cell epitopes, which have to be improved in order to achieve a complete response and control the induction of Tregs with no induction of other mechanisms, thus inducing desensitization and enabling treatment to be given with a high degree of safety.

New treatments during coming years will also play an important role, complementing SIT. Nonspecific IT is becoming more relevant and many studies are being developed. Herb formulas are undergoing human trials and show promising results. The use of recombinant monoclonal humanized anti-IgE is being considered as a treatment for FA. Anti-IgE therapy has also been applied in combination with IT to induce a low IgE reactivity and to reduce the adverse effects of IT, showing a good safety profile.

The current strategies applied to deal with FA are able to induce a state of desensitization in a high percentage of cases, but very few reports have demonstrated the induction of tolerance,

Executive summary

- Food allergy (FA) is an increasingly common disorder in western countries, with no curative therapies at present. FA has a prevalence of around 6% in children and 4% in adults.
- Diagnosis of FA is based on clinical history and an IgE-detection test with no optimal sensitivity and inability to differentiate either cross-reactivity or the severity of the reaction. New techniques such as the basophil activation test, component-resolved diagnosis and the use of epitopes could improve the diagnosis, although more studies are required to establish their correlation with *in vivo* tests such as oral food challenges.
- Treatment of FA consists of strict avoidance of the known or suspected food. However, specific immunotherapy has been shown to induce long-term desensitization with immunological changes associated with improvement of the symptoms and a reduction in drugs required.
- Sublingual and oral immunotherapies appear to be a promising approach for the treatment of FA, although they are not currently used in routine practice.
- The development of modified recombinant proteins and T-cell epitopes may also be a tool in FA treatment, since they are able to stimulate the proliferation of specific T cells without inducing an IgE response.
- Nonspecific immunotherapies in FA have gained interest. The use of new products based on Chinese herbal formulas and humanized anti-IgE treatments that treat multiple allergies at the same time are under development, although clinical trial results are still required to evaluate their safety and efficacy.
- The achievement of permanent tolerance is the goal of allergen IT and efforts should focus on revealing the associated immunological mechanisms. More controlled studies, with a higher power and using standardized protocols, are required to accurately determine safety and efficacy.

as a permanent desensitization, allowing subjects to ingest the allergens *ad libitum*. The achievement of permanent tolerance is the goal of allergen IT and efforts should focus on revealing the immunological mechanisms associated with FA, which in turn will improve allergen IT or generate new approaches. During the coming years, protocols and allergens have to be standardized and refined in order to be able to compare the results of different studies and to obtain relevant and more precise data about the prevalence, safety profile and efficacy of IT in FA.

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