



New pharmaceutical approaches for the treatment of food allergies

Ana Brotons-Canto, Nekane Martín-Arbella, Carlos Gamazo & Juan M. Irache

To cite this article: Ana Brotons-Canto, Nekane Martín-Arbella, Carlos Gamazo & Juan M. Irache (2016): New pharmaceutical approaches for the treatment of food allergies, Expert Opinion on Drug Delivery, DOI: [10.1080/17425247.2016.1247805](https://doi.org/10.1080/17425247.2016.1247805)

To link to this article: <http://dx.doi.org/10.1080/17425247.2016.1247805>



Accepted author version posted online: 12 Oct 2016.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)

Publisher: Taylor & Francis

Journal: *Expert Opinion on Drug Delivery*

DOI: 10.1080/17425247.2016.1247805

New pharmaceutical approaches for the treatment of food allergies

Ana Brotons-Canto¹, Nekane Martín-Arbella¹, Carlos Gamazo², Juan M. Irache*¹

Affiliation

¹Department of Pharmacy and Pharmaceutical Technology, University of Navarra,
31008-Pamplona, Spain

²Department of Microbiology, University of Navarra, 31008-Pamplona, Spain

***Corresponding author:**

Juan M. Irache

Dep. Pharmacy and Pharmaceutical Technology

University of Navarra

C/ Irunlarrea, 1

31080 – Pamplona

Spain

Tel: +34948425600

Fax: +34948425619

Email: jmirache@unav.es

Abstract

Introduction: Allergic diseases constitute one of the most common causes of chronic illness in developed countries. The main mechanism determining allergy is an imbalance between Th1 and Th2 response towards Th2.

Areas covered: This review describes the mechanisms underlying the natural tolerance to food components and the development of an allergic response in sensitized individuals. Furthermore, therapeutic approaches proposed to manage these abnormal immunologic responses food are also presented and discussed.

Expert opinion: In the past, management of food allergies has consisted of the education of patients to avoid the ingestion of the culprit food and to initiate the therapy (e.g. self-injectable epinephrine) in case of accidental ingestion. In recent years, sublingual/oral immunotherapies based on the continuous administration of small amounts of the allergen have been developed. However, the long periods of time needed to obtain significant desensitization and the generation of adverse effects, limit their use.

In order to solve these drawbacks, strategies to induce tolerance are being studied, such as the use of either adjuvant immunotherapy in order to facilitate the reversion of the

Th2 response towards Th1 or the use of monoclonal antibodies to block the main immunogenic elements.

Keywords: food allergy, allergens, immunotherapy, nanoparticles, adjuvants, monoclonal antibodies, desensitization, tolerance

Article highlights box

- Food allergies are increasing in prevalence, lack current curative therapies, and can be life-threatening. In general, they result from immune deviation toward a Th2 response, where IL-4, IL-5 and IL-13 induce IgE antibodies that mediate the allergic reaction
- Traditional management of food allergies is associated with a strict avoidance of the culprit food and specific training on the recognition of unexpected reactions and their management
- Allergen immunotherapy aims to stimulate a Th1 immune response or generate suppressive (regulatory) T cells, resulting in down-regulation of Th2 responses. However, conventional immunotherapy (sublingual, oral or epicutaneous) is associated with tedious protocols and daily doses for long periods of time
- The use of nanoparticles as allergen delivery systems for mucosal immunotherapy may be an adequate strategy to develop more effective and safer treatments, diminishing the allergen exposure to IgE and promoting Th1 and/or T-regulatory responses particularly when related to PAMPs (CpG motifs, LPS)

- The therapy based on monoclonal antibodies is designed to target circulating IgE or specific cytokines (e.g. IL-5) and hence, block the generation of an allergic reaction in the presence of the allergen

Abbreviations

EAACI: European Academy of Allergy and Clinical Immunology

FA: food allergies

PP: Peyer's patches

FAE: follicle-associated epithelium

GALT: gut-associated lymphoid tissue

PRR: pattern-recognition receptors

TLR: Toll-like receptor

APC: antigen presenting cells

DC: dendritic cells

SR: self-report

SPT: skin prick test

OFC: oral food challenge

DBPCFC: double-blind placebo-controlled food challenge

sIgE: specific IgE

MA: meta-analysis

SIT: subcutaneous immunotherapy

SLIT: sublingual immunotherapy

OIT: oral immunotherapy

EPIT: epicutaneous immunotherapy

PAMPS: pathogen associated molecular pattern

LPS: lipopolysaccharide

PLGA: poly(DL-lactide-co-glycolide)

OVA: ovalbumin

1. Introduction

Adverse reaction to foods is a broad term referred to any abnormal response associated with food ingestion. Based on the pathophysiological mechanisms involved, these adverse effects may be subdivided into either food intolerance or food allergy [1]. Food intolerance refer to a physiologic adverse response due to the presence of toxins and pharmacologically active components (e.g. vasoactive amines, caffeine or salicylates), idiosyncratic responses (e.g. sulfite-induced asthma) or, most commonly, secondary to metabolic disorders (e.g. lactase or fructose deficiencies) [2].

Otherwise, food allergy is defined as a reproducible effect mediated by any abnormal immunologic response to a food in a susceptible host. The European Academy of Allergy and Clinical Immunology (EAACI) has estimated more than 17 million people in Europe would be affected by at least one food allergy. Furthermore, 3.5 million of these patients (21%) would be younger than 25 years [3].

From an immunological point of view, food allergies (FA) are mainly mediated by IgE [4]. IgE is a unique class of immunoglobulin involved in an immediate allergic reaction (hypersensitivity class I) after allergen exposure. This reaction is implicated in the pathogenesis of many cases of asthma, rhinitis, urticaria, atopic dermatitis, anaphylaxis and gastrointestinal adverse food reaction [5]. In allergic individuals, IgE-mediated episodes are the classic immunological reactions against a food allergen and appear, usually, after the exposure to one or more specific foods [4]. In contrast, non-IgE-mediated reactions, are poorly defined, and are believed to be T-cell-mediated. Their onset is delayed (after the ingestion of the offending food) and they may produce enterocolitis syndrome, dietary protein enteropathies and dietary induced proctocolitis [6, 7].

This review focuses on the approaches proposed to treat IgE-mediated FA. Furthermore, an updated overview of the mechanisms underlying the natural tolerance to food components and epidemiological data is also provided.

2. Physiological and Immunological considerations

2.1. The gut mucosa

The gastrointestinal tract encompasses the largest surface area in the human body. Its main function is to process ingested food into a form that can be absorbed and used in metabolic pathways, but, at the same, to prevent the penetration of harmful pathogens into the body [8]. For this purpose, a single-cell layer of columnar intestinal epithelial cells, joined by tight junctions and covered with a thick mucus layer that traps particles and microorganisms, conforms the so-called “gastrointestinal mucosal barrier” (Figure 1). Although the majority of cells bordering the intestinal lumen are absorptive enterocytes, which are adapted for metabolic and digestive functions, other specialized cells (e.g. enteroendocrine cells, goblet cells and Paneth cells) are also present [9]. Under this single layer of cells, there is a stroma of loose connective tissue populated by lymphocytes. Besides, in some certain parts of the small intestine there is an increase in the amount of mucosal lymphoid tissue, which forms conspicuous clusters of lymph nodules called Peyer's patches (PP) that extend through the submucosa. PPs are surrounded by the follicle-associated epithelium (FAE) that forms the interface between the gut-associated lymphoid tissue (GALT) and the luminal microenvironment. The FAE contains specialized cells named M cells (for microfold) that transport luminal components (e.g. dietary proteins and antigens) and bacteria toward the underlying

immune cells, which inhibit or activate inhibit the immune response leading to either tolerance or systemic immune cell responses [10].

Gel-forming mucins, the major components of mucus, are continuously secreted by goblet cells, scattered along the gut epithelium, in order to maintain the properties of the mucus gel barrier. In addition, luminal and brush border enzymes and bile salts, as well as the changing pH conditions along the gut, serve to digest food, destroy pathogens and render antigens and allergens less immunogenic. All of these elements, along with peristalsis, belong to the non-immunological mechanisms of innate immunity, whereas the immunological components include the complement system, phagocytes and natural killer cells, among others [11]. On the other hand, the adaptive immune components include intraepithelial and lamina propria lymphocytes, PP as well as antibodies (e.g. secretory IgA) and cytokines [12]. At the interface of innate and adaptive immunity there are the Pattern-Recognition-Receptors (PRRs) (e.g. Toll-like receptors, TLRs), which are proteins expressed on/in cells of the innate immune system and have a major role in down-regulating or enhancing immune responses. After recognition of microbial pathogens, TLRs trigger induction of inflammatory cytokines and chemokines [13]. TLR signaling also up-regulates costimulatory molecules on specialized antigen presenting cells (APCs), such as dendritic cells (DCs), essential for inducing an specific adaptive immune responses [13].

2.2. Oral Tolerance and Immune response to food

FA are mainly caused by dietary proteins. Generally, dietary proteins and other food components are chemically and enzymatically degraded in the stomach and intestine. However, in some cases, some segments or epitopes of these proteins resist the

digestive processes within the gut and may induce a hypersensitivity reaction after interacting with mucosal immune system. These epitopes (or allergens) share some features: they are water-soluble glycoproteins, from 10 to 70 kDa in size, and relatively stable to heat, acid, and proteases [4]. The interaction or contact between the allergen and the immune system may occur in different ways. Thus, some DCs project “dendrites” that penetrate the epithelium to sample luminal antigens/allergens [8]. In a similar way, M cells overlying PP can take up particulate allergens and deliver them to sub-epithelial DCs [8]. Soluble proteins cross the epithelium through transcellular or paracellular routes to encounter T cells or macrophages in the lamina propria [14]. Finally, dietary proteins can be also taken up by intestinal epithelial cells that would act as non-professional APCs, given that they constitutively express MHC molecules on their basolateral membranes [15] and can present antigen to primed T cells (Figure 1).

Oral tolerance, or the state of local and systemic immune unresponsiveness, is produced by an active suppression by regulatory T cells and/or a clonal anergy (deletion). Regulatory T cells suppress immune responses through soluble or cell surface-associated down-regulatory cytokines (e.g. IL-10, and TGF- β) [8] (Figure 2). On the other hand, anergy can occur through T-cell receptor ligation in the absence of co-stimulatory signals provided by soluble cytokines (e.g. IL-2) or by interactions between receptors on T cells (CD28) and counterreceptors on APCs (CD80 and CD86) [16]. Clonal deletion occurs by means of FAS-mediated apoptosis, which can be blocked by the proinflammatory cytokine IL-12 [17].

When a food allergen (that has survived to digestion) pass through the epithelial barrier, it may be presented by APCs. In some individuals, and for reasons still not well

understood, the presentation of the antigen to T-cells in presence of IL-4 starts a differentiation to Th2. These Th2 cells produce more IL-4, IL-5 and IL-13 that encourage the production of IgE. This first contact with the allergen is called “sensitization”. Then, and after a new exposure to the antigen (elicitation phase), allergen become attached to the FcεRI on the surface of effector cells (mast cells, eosinophils and basophils), triggering the rapid release of proinflammatory mediators, generally within minutes, including histamine and leukotrienes that cause the symptoms of allergy [18] (Figure 2).

Food allergy likely results from either a failure in establishing oral tolerance or a breakdown in existing tolerance. In the former, the maturity of both immunological and mechanical barriers is an important factor preventing the development of FA. Therefore, in some cases, children between 2-7 years “outgrow” the allergy without any treatment due to mucosal maturation [19]. In the later, in a susceptible host, a failure to develop or a breakdown in oral tolerance may result in sensitization to food allergens [20]. Thus, decreased gastric acidity caused by potent antacids can lead to increased sensitization in both children and adults [20]. In a similar way, altered intestinal permeability leading to increased exposure to intact proteins might promote sensitization and might enhance the severity of food-induced allergic reactions [4].

3. Diagnosis, prevalence and economic burden

FA may cause from acute and relatively mild symptoms to severe, life-threatening reactions (anaphylactic shock), which require immediate medical treatment. FA most commonly affects the skin (atopic dermatitis, urticaria, angioedema, eczema and various skin rashes) [4]. In parallel, frequent gastrointestinal manifestations are also associated

with cutaneous symptoms, including dyspepsia, diarrhea, gastroesophageal reflux, oral pruritus and angioedema [4]. In some cases, symptoms may be triggered in the respiratory tract (e.g. laryngeal edema, rhinorrhea, bronchospasm, dyspnea and nasal congestion), and/or the cardiovascular system (e.g. tachycardia and hypotension) [21]. Fortunately, anaphylactic shock is much less frequent than skin rashes or symptoms in the gastrointestinal tract.

The variety, pattern and threshold of these symptoms turn difficult the diagnosis of the food allergy. Therefore, the diagnosis has to be based on strict, well-defined food elimination and challenge procedures establishing a causal relation between the ingestion of a particular food (or food protein) and a subsequent obvious clinical reaction [22]. The first step is always the patient's history aimed at identifying the suspected relevant food(s) and the relationship between the ingestion of a specific food and the occurrence of symptoms. Then, the dependence of clinical manifestations from an immune mediated reaction must be assessed. For this purpose, skin prick test (SPT) and specific IgE (sIgE) determinations are usually used [23]. SPT is a well-standardized, simple, cheap, high negative predictive value (>90%) and low-risk diagnostic test. However, the SPT to foods have a low specificity with a low positive predictive value [23]. Thus, a positive result cannot be considered as a definitive diagnosis [23].

The quantitative methods used nowadays to determine sIgE show high levels of sensitivity (and negative predictive values), with high specificity and positive predictive value [24]. Specific IgE (sIgE) levels exceeding a certain value ("diagnostic cut-off") are considered as a predictive value for a symptomatic allergy [25]. Thus, in the presence of a compatible clinical history, sIgE can confirm the diagnosis of FA without

requiring further challenge tests. Nevertheless, patients with poly-sensitization and pollen-food syndromes, frequently require oral provocation tests (OPT) for a correct identification of the sensitization profile [26]. These tests consist of gradually increasing doses of the appropriately diluted food, starting from the lowest dose and checking the presence of relevant symptoms. Ideally these OPT should be double-blind placebo-controlled food challenge (DBPCFC). Apart these conventional diagnostic tools, other complementary tests (i.e, the atopy patch test, the basophil activation test and electrodermal tests) have also been proposed [23].

In spite of the fact that FA can arise to any food, more than 85% of these disorders are related to the ingestion of milk, egg, peanut, tree nuts, shellfish, fish, wheat, sesame seed and soy [27]. Allergies to milk, egg, soy and wheat, are usually outgrown within the first ten years of life [27]. In contrast, allergies to peanut, tree nuts, fish, and shellfish are often lifelong disorders. Peanut and tree nuts are responsible for the most serious allergic reactions and food-allergy related fatalities [28]. Tables 1-3 summarize some studies reporting the prevalence for different FA. For peanut allergy, the prevalence is around 2% (Table 1) for general population; although, children with atopic disorders would tend to have a higher incidence [29]. Studies have also shown that children with atopic dermatitis and food allergy have a higher prevalence of allergic rhinitis and asthma [25]. For shellfish (Table 2) and cow's milk allergy (Table 3), the pooled estimate of prevalence is close to 0.5-0.6% when SPT and/or OFC were used to calculate these values. In case of tree nut, the prevalence rate would be in the range between 0.4 and 1.4% of the overall population [30]. Finally, fruit and vegetables allergies would affect to a 0.4-6.6% and 0.5-2.2% of the population, respectively [30].

In spite of the high incidence of FA, few studies describe their economic impact. Initially, two types of costs may be considered: the direct medical cost (office visits, rescue medication, visits to emergency department, hospitalization, medicines...) and the indirect costs such as the need of special foods or the loss of productivity. In case of children, these indirect costs might be higher due to the need of caregivers. In 2007, for an estimated prevalence in children of 5-6% and 3.7% in adults, the direct medical costs due to FA were estimated by Patel and collaborators around \$300 million whereas the indirect costs accounted for \$200 million [44]. Office visits reported for 52.5% of costs, and the remainder was split between emergency visits (20%), inpatient hospitalizations (11.8%), outpatient visits (3.9%), ambulance runs (3%), and epinephrine devices (8.7%) [44]. In a similar way, a European study of 2013 showed that food allergic children suffered from four more annual visits to health care professionals than healthy children. These annual direct medical cost were estimated to be 1,334\$ per child [45]; although the indirect costs, especially those borne by families, were not quantified. In a very recent study, Gupta and collaborators did a comprehensively quantification of the economic impact of childhood food allergy in US (using a prevalence of 8%). Overall, the food allergy cost was calculated to be \$24.8 billion annually or \$4184 per child. The cost borne by families was estimated in \$20.5 billion [46].

4. Treatment

For a long time, the only available management in FA was the strict avoidance of the culprit food and its substitution, if possible, by hydrolysed formulas or artificial supplements containing the essential nutrients [22]. However this nutritional intervention approach is risky and has to be complemented with training on the

recognition of unexpected reactions and their management, including (in some cases) the administration of self-injectable epinephrine [47]. In spite of this, severe accidental reactions are frequent owing to the ubiquitous use of some of these allergens (especially peanut, milk and egg). Nevertheless, in the last years, important efforts to develop effective methods to protect food allergic individuals from severe reactions and potentially cure them are underway. These new approaches include allergen-specific therapies and immunomodulatory treatments. The immunomodulatory treatments, also known as non-allergen specific therapies include, among others, the use of anti-IgE antibodies to interrupt inflammatory signals. The allergen-specific therapies are based on the administration of increasing doses of the culprit food (or allergen) or on the induction of tolerance by the association of the allergen to specific adjuvants.

4.1. Allergen-specific therapies

The allergen specific therapies aim to alter the allergic response to the allergen without triggering an adverse immune response to the therapy itself. If the therapy is effective, ideally, the food can be consumed in normal quantities with no symptoms. In principle different approaches may be identified, including the use of immunotherapy with standard food allergens, immunotherapy with hypoallergenic mutants and the use of adjuvants to facilitate the delivery and presentation of the allergen (proteins or plasmid DNA) to APCs in order to induce tolerance.

4.1.1. Allergen immunotherapy

Allergen immunotherapy is based on the continuous administration of small amounts of the allergen in order to provide protection against the allergic symptoms and

inflammation associated with the natural exposure to the allergen [48]. In food allergy, immunotherapy may result in desensitization or tolerance to the specific allergen. The term “desensitization” refers to a temporary and reversible state, in which patients are able to eat a higher allergen dose without any symptom during treatment when compared with pre-treatment tolerated dose [5]. In contrast, sustained unresponsiveness (immunological tolerance) is the ability to ingest the allergen without adverse reaction, some weeks after stopping receiving therapy. Different routes of administration have been investigated, including subcutaneous, sublingual, oral and epicutaneous routes. In all cases, immunotherapy tries to reduce the ratio Th2/Th1 and induce T-regulatory effector cells. These changes are reflected by an increased production of IL-10 by APCs and other immune cells that, along with TFG-beta (Figure 2) induce a class switching B cells to IgG4 and IgA, and a suppression of IgE production [49]. In addition, IgG4 and IgA compete with IgE for allergen binding, decreasing the allergen capture by basophiles [50].

4.1.1.1 Subcutaneous immunotherapy (SIT)

SIT, as other immunotherapeutic approaches, involves long and tedious protocols such as weekly injections during a build-up phase, followed by monthly maintenance injections for a period of 3–5 years [51]. Although efficacy of SIT has been demonstrated (e.g. hay fever and hymenoptera sensitivity), for FA entails the risk of systemic anaphylactic reactions [52]. In fact, different studies have been recorded significant adverse events (with, in general, modest clinical improvements), suggesting that SIT may be an unsuitable treatment modality for FA [53].

4.1.1.2 Sublingual immunotherapy (SLIT)

SLIT involves the administration of small drops of an allergen extract (in doses ranging from micrograms to milligrams) under the tongue, which is held for 2 minutes and then swallowed [54]. SLIT ameliorates clinical symptoms in a variety of IgE-mediated respiratory diseases, including asthma and rhinoconjunctivitis [55]. This fact would be related with the direct activation of Langerhan's cells in the oral mucosa, which are protolerogenic, leading to down-regulation of the allergic response [56].

SLIT protocols for the treatment of food allergy typically comprise two phases: a build-up phase consisting of weekly to biweekly dose escalations administered until a target dose is reached followed by a second maintenance phase in which daily home dosing over the course of months or years is administered [57]. In the maintenance regimen, the daily doses of the allergen are often within the range of 2–7 mg protein [54, 58].

In spite that there are few studies of SLIT, hopeful results for the treatment of patients with several common FA have been obtained, including peanut [59], milk [60], hazelnut [61], and peach [62] allergies. Regarding hazelnut allergy in adults, a randomized double-blind placebo-controlled trial showed that, after 5 months of treatment, SLIT increased the threshold of patients to this nut from 2.3 g to 11.6 g without allergic symptoms after an OFC [63]. In another interesting study with peanut allergic children, who underwent 6 months of dose escalation followed by 6 months of maintenance dosing, the treatment permitted to safely ingest 20 times more peanut protein compared to the placebo group. This fact was correlated with a decrease in skin prick test wheal size and decreased basophil responsiveness after stimulation with peanut. However, no changes were observed in the percentage of regulatory T cells, or IL-10 and IFN- γ production [59]. Moreover, SLIT treatment of peach allergic patients during 6 months increased up to 9-fold their tolerability to the fruit [62].

4.1.1.3 Oral immunotherapy (OIT)

OIT usually involves the daily and gradual administration of small amounts of an allergen mixed with a food vehicle over the course of several months to years [54]. Unlike other routes of food allergen-specific immunotherapy, OIT utilizes the pathways underlying oral tolerance that are the physiologic response to ingested food protein antigens [63].

In general, OIT protocols comprise a first step in which an initial rapid dose escalation (e.g. 6-8 doses of the allergen in 1-2 days) followed by a build-up phase of about 6-12 months till a target dose is reached. Afterwards, a maintenance phase during several months or years is conducted at home [58]. Doses of food allergens in OIT are significantly higher than those used in SLIT protocols [54]. The permanence of protection is usually tested with the intentional interruption of OIT dosing for at least 4–12 weeks followed by a ‘tolerance’ food challenge [64].

For cow’s milk allergy, the rates of desensitization after OIT range from 36% to 77% with an estimate of tolerance close to 30% [65]. In a MA of studies with milk allergic patients, desensitization was 10 times more likely in OIT-treated than in non-OIT-treated individuals [65]. For egg allergy, the estimated desensitization interval is between 75-100% with tolerance rates close to 30% [66, 67]. Regarding peanut allergy, results and success rates are highly variable with good figures for desensitization but very modest values when tolerance is measured. Thus, in a long-term study of peanut OIT, 24 subjects were treated up to 5 years with maintenance daily dose with 4 g of peanut protein [68]. One month after stopping OIT, only 50% passed a challenge and were considered to have achieved sustained unresponsiveness and added unrestricted peanut to their diet. With a shorter duration of peanut OIT, after two years of daily

dosing with 4 g of peanut protein, 87% became desensitized [69]. However, after 3 months of strict peanut elimination, only 30% of patients passed the peanut challenge and, following an additional 3 months period of peanut avoidance, only 13% remained tolerant during the final peanut challenge.

Comparing OIT with SLIT, in general, available evidences suggest that SLIT is less effective than OIT in inducing desensitization, but provides a better safety profile [60].

However, up to date, no adequately controlled and rigorous trials of OIT (or SLIT) have demonstrated the development of permanent tolerance (or even long-term desensitization) due to therapy as opposed to natural acquisition [70].

4.1.1.4 Epicutaneous immunotherapy (EPIT)

EPIT involves the application of an allergen-containing patch to the skin surface in order to activate the skin Langerhans cells [58] and, hence, promote the down-regulation of effector cell responses. The use of the epicutaneous route for allergen immunotherapy has been facilitated by the development of Viaskin® (DBV Technologies, France), which allows the allergen to be applied repeatedly onto the intact skin at home for a long-lasting contact but without any injection or swallowing of the allergen [71]. This device is a polyethylene electrically charged system containing the allergen that is slowly released from the patch to the skin allowing reaching epidermal immune system without any risk of massive transcutaneous passage [71].

A proof-of-principle study for use of EPIT in patients with food allergy was conducted in a 3-month pilot in 18 children presenting milk allergy [72]. Treatment consisted of three 48-hour patch applications on the back (using skimmed milk powder as active substance) per week for 3 months. Adverse events were mostly local erythema/eczema occurring at application sites and remaining visible for several days; whereas the

cumulative reactive dose of milk increased about 10 times in the treatment group at the end of the study. In another study with peanut allergic children who receive a patch for 18 months, the responder rate (defined as at least a 10-fold increase in cumulative reactive dose from baseline) was estimated to be close to 70% [73]. Apparently, EPIT presents a safer profile than other current immunotherapies.

However, EPIT is strongly dependent on skin condition. Thus, in animal studies, it has been shown that EPIT in intact skin decreases specific IgE levels and increases specific IgG2a antibodies (Th1 response). On the contrary, when EPIT is applied on stripped or wounded skin, the specific IgE levels in the plasma of animals significantly increases whereas the amount of IgG2a remains constant [74].

4.1.2. New approaches for allergen immunotherapy

With conventional allergen immunotherapy, the need for daily dosing raises concerns regarding patient adherence over long periods of time, as well as unintentional dosing interruptions due to illness or travel. Moreover, adverse reactions are frequent during the treatment and in some cases are unacceptable for clinical standard practice. Considering these concerns, new immunotherapy methods are being considered in order to increase the risk-to-benefit ratio and to recuperate the confidence of the patients at the beginning and, later to continue with the treatment. In this context, the synthesis of new antigens with reduced allergenicity (e.g. mutated recombinant proteins) may be an interesting approach. The use of mutated recombinant proteins instead of native food allergens may be adequate to avoid the activation of mast cells during specific allergen immunotherapy. The objective is to identify IgE binding sites on proteins and modify these sites to ablate IgE binding, while preserving their ability to stimulate T cells. This

strategy has been successfully used to generate hypoallergenic mutants of peanut [75], fish [76] and apple allergens [77].

Another interesting strategy would be the use of “adjuvanted” immunotherapy. In fact, the association of allergens with mucosal adjuvants has been proposed in order to develop more effective and safer immunotherapy treatments. In a simplistic approach, depending on their mode of action, adjuvants may be classified into two different groups; although, some compounds can act by both mechanisms simultaneously. The former would include those adjuvants that act as delivery systems facilitating the uptake and presentation of the antigen/allergen by the APCs [78]. The latter would include the immunomodulatory agents that specifically bind to the PRRs on the host cells (e.g. TLRs), triggering their activation [50]. Among others, this group of immunopotentiators includes the so-called “pathogen associated molecular patterns” (PAMPs) such as the lipopolysaccharide (LPS) from Gram-negative bacteria and their derivatives (e.g. monophosphoryl lipid A), flagellin and CpG motifs.

Regarding the use of adjuvants in food SLIT and OIT, these compounds should be adapted to the anatomic and physiological characteristics of the gut. Thus, these adjuvants should avoid the mucosal physiological barriers and gain access to the epithelium through the intricate network of glycoproteins covering their targets [50]. Once there, these mucosal adjuvants should facilitate the antigen uptake by APCs and, ideally, stimulate and/or promote a Th1 immune response without inducing autoimmunity [78]. For these purposes, particulate delivery systems based on polymers and macromolecules may be a good strategy. In fact, these devices constitute a group of delivery systems with interesting abilities as adjuvants for both conventional and mucosal vaccination, since they can enhance the delivery of the loaded antigen to the

gut lymphoid cells, due to their ability to be captured and internalized by cells of the GALT [50]. Furthermore, nanoparticles may be used to load the biologically active material (e.g. allergens) offering the following advantages: (i) protection from hydrolysis and/or enzymatic degradation, (ii) controlled and sustained release properties of its cargo, and (iii) co-encapsulation of the allergen and co-immunostimulatory molecules (e.g. CpG motifs or LPS). In addition, these polymer nanoparticles may be, in general, easily tailored in order to improve their mucus-penetrating properties and/or targeting abilities. Among others, nanoparticles based on poly(DL-lactide-co-glycolide) (PLGA), chitosan or poly(anhydrides) have given encouraging results for mucosal allergen delivery.

Therefore, Pecquet and collaborators were the first to propose the use of PLGA microparticles to elicit oral tolerance against an allergen [79]. In their work, they demonstrated that the oral administration of β -lactoglobulin encapsulated in PLGA particles elicited a greater oral tolerance than the free protein. In fact, the tolerogenic efficient dose was reduced 10,000 times, compared to oral administration of soluble lactoglobulin [79]. In another interesting work, the possibility of increasing the capability of PLGA nanoparticles to promote Th1 responses was evaluated by their combination with CpG sequences [80]. CpG motifs provide steric hindrance to reduce IgE binding and enhance Th1 responses by stimulation of TLR9, APCs and NK cells to secrete gamma-interferon (IFN- γ) and IL-12 [81]. Thus, the oral administration of PLGA-CpG nanoparticles containing a peanut extract during 4 weeks to laboratory animals provided a long-lasting protection from anaphylaxis to repeated challenge. During this period, animals treated with the nanoparticles displayed lower levels of

specific IgE, IgG1 (Th2 marker) and pro-Th2 cytokines (IL-4, IL-5 and IL-13) than controls [80].

Another interesting class of nanocarriers for allergen oral delivery are those based on the copolymer of methyl vinyl ether and maleic anhydride (Gantrez AN®). Interestingly, these poly(anhydride) nanoparticles induced innate immune responses mediated by TLR-2 and TLR-4 dependent manner and maturation of DCs with a significant up-regulation of CD40, CD80, and CD86 and a biased Th1 present response in animal models [82]. This is an important finding since it has been recently shown that the use of multiple TLR-agonists influence the induction of long-term memory cells [83].

One single dose of these nanoparticles, using ovalbumin (OVA) as a food allergen model and orally administered to mice, induced high serum levels of both OVA-specific IgG2a (Th1 marker) and IgG1 (Th2 marker) levels as well as high levels of IgA and IL-10 [84, 85]. Moreover, these nanoparticles were able to decrease the anaphylactic symptoms of sensitized animals and to protect them from death after a challenge with OVA administered intraperitoneally, in contrast to the control group [84]. Interestingly, the co-encapsulation of LPS in these OVA-loaded poly(anhydride) nanoparticles, but not its binding to the nanoparticle surface, also protected sensitized mice from the challenge [84].

In another work using a peanut protein extract, similar poly(anhydride) nanoparticles were prepared and orally administered to laboratory animals. After one single dose, nanoparticles induced a balanced Th1 and Th2 antibody response, accompanied by a low specific IgE induction. Furthermore, the levels of pro-Th2 cytokines (IL-4, IL-5,

IL-6) were lower compared to control, whereas mice treated with the nanoparticles containing the peanut extract displayed high levels of IFN- γ and IL-10 [86].

Another interesting material for the preparation of nanoparticulate adjuvants is the cationic polysaccharide chitosan. Firstly, chitosan is capable of driving potent cell-mediated immunity, promoting DC maturation by inducing type I interferons and enhancing antigen-specific T helper 1 (Th1) responses in a type I IFN receptor-dependent manner [87]. Secondly, nanoparticles based on chitosan possess bioadhesive properties prolonging their residence within the mucosal surface. Thirdly, its cationic character is ideal to design devices for the delivery of polyanions, such as DNA or RNA [88]. In this context chitosan nanoparticles were proposed for the oral delivery of a pDNA encoding for Arah2, a dominant peanut allergen. Compared with non-immunized mice or mice treated with 'naked' DNA, animals immunized with nanoparticles showed a substantial reduction in allergen-induced anaphylaxis associated with reduced levels of IgE, plasma histamine and vascular leakage [88].

4.2. Non-allergen specific therapies

In this group a variety of different procedures have been proposed including the use of Chinese herbal therapies [89], engineering lactic acid bacteria [90], and helminthic therapies [91]. However, the more interesting approaches would be those based on the use of monoclonal antibodies and probiotics. In principle, their main advantage is that they would allow for the treatment of multiple FA in a single patient with one therapy.

4.2.1 Monoclonal antibodies

In the last years different monoclonal antibodies have been proposed as therapeutic tools to specifically target and block the main actors involved in the generation of an allergic reaction. Table 4 summarizes some of those monoclonal antibodies that would be of interest for treating FA. Two of these monoclonal antibodies (omalizumab and talizumab) present a similar mechanism of action. Omalizumab, a humanized recombinant IgG1 antibody, binds Fc portions of circulating IgE and reduce cell surface levels of IgE due to the binding to FcεRI on cell surface [92, 93]. Given its mechanism of action, recent reports have suggested its potential clinical use for the effective treatment of food allergy and some forms of anaphylaxis [94] as well as to reduce side effects of some OIT [93].

In another interesting study, talizumab (TNX-901) was evaluated in peanut allergic patients. TNX-901 induced an important increase in the threshold dose on peanut challenge eliciting allergic reactions from a mean of 90 mg to 1,400 mg of peanut protein [95]. Another interesting biological that may block the generation of an allergic reaction is mepolizumab that specifically binds IL-5 [96]. This monoclonal antibody has been recently approved for the treatment of patients presenting severe asthma with an eosinophilic phenotype [97]. Finally, lumiliximab, a primatized macaque/human anti-CD23 monoclonal antibody is capable of reducing the IL-5 allergen-mediated secretion by Th2 cells and mast cells [98]. Figure 3 shows the mechanism of these monoclonal antibodies in the allergic reaction induced by a food allergen.

4.2.2. Probiotics

Several studies have shown the immunomodulatory effects of probiotic bacteria. Thus, certain strains of lactobacilli and bifidobacteria would inhibit the synthesis of Th2

cytokines and would act as potent inducers of IL-10 production in different peripheral blood mononuclear cell cultures [90]. In vivo, the oral administration of a probiotic mixture containing 8 different probiotic strains displayed beneficial effects in OVA-sensitized mice, with a reduction in symptoms severity and a down-regulation of Th2 cytokine mRNA expression, in particular IL-13, IL-4 and IL-5 [101]. In another study, the oral administration of *Bifidobacterium lactis* and *Lactobacillus acidophilus* to OVA sensitized mice decreased the serum levels of OVA-specific IgE, IgG1 and IgA. Additionally, the level of IL-4 was significantly lower, and the levels of IFN- γ and IL-10 were significantly higher in the sensitized mice treated with probiotics than those observed in controls [102]. Other authors showed that the oral administration of *Lactobacillus rhamnosus* GG in atopic children resulted in elevated IL-10 concentrations, indicating that specific probiotics may have anti-inflammatory effects and, possibly, they would also enhance regulatory or tolerance-inducing mechanisms [103]. In peanut allergic patients, the co-administration of *Lactobacillus rhamnosus* as adjuvant improved the efficacy of the OIT treatment [104].

5. Conclusions

In summary, FA, defined as an adverse immune response to food proteins, affect to an increased percentage of population. Currently, management of FA consists of educating the patient to avoid ingesting the responsible allergen and initiating therapy if accidentally this ingestion occurs. In the last years novel therapeutic approaches have been proposed including allergen-specific immunotherapies (oral, sublingual or cutaneous administration) and immunomodulatory treatments (e.g. monoclonal antibodies, probiotics, etc.). Allergen immunotherapy involves the administration of

daily small amounts of an allergen over long periods of time (up to some years), that compromise the adherence of patients to the treatments. In addition, these immunotherapy treatments would not offer adequate tolerogenic immune responses. In order to solve these drawbacks, the association of allergens with nanoparticulate adjuvants would be an adequate approach to develop more effective and safer treatments. Thus, nanoparticles can be designed to reach the gut epithelium and, taken into account their inherent inclination to be naturally captured by APCs, facilitate the development of tolerance by promoting strong and lasted Th1 responses. On the other hand, immunomodulatory treatments based on monoclonal antibodies have been proposed in order to block the allergic response. Nevertheless, in spite of the significant progress developed in the last years, there is still much to be done to offer these new approaches to food allergy patients.

6. Expert Opinion

Food allergy is an immune-based disease that has become a serious health concern that affects children and adults and seems to be increasing in prevalence. As the most common food allergens are highly prevalent in the diet, patients and their families must stay alert. Surprisingly, despite the risk of severe allergic reactions (e.g. anaphylaxis) and even death, the disease was only managed by dietary modifications, education, and behavioral approaches to avoid allergens as well as pharmacological management of the symptoms in urgent cases. This absence of adequate treatments contrast with the success of therapies for other allergic diseases such as asthma, allergic rhinitis and insect-venom anaphylaxis, for which different types of drugs (e.g. steroids, bronchodilators or vasoconstrictors) and immunotherapy are highly effective.

In the last years, allergic-specific therapies have been proposed for desensitization and, eventually, induce tolerance by sublingual, oral or epicutaneous routes of administration. All of these immunotherapeutic treatments are based on the administration of increasing doses of the allergen in order to reach a target dose. Then the allergic patient receives daily the maintenance dose for long periods of time (from months to years). As a consequence, the antibody isotype would change from IgE to IgG4 inducing desensitization. However, this conventional allergen immunotherapy is completely dependent on the patient motivation and her/his adherence over long periods of time without interruptions. In addition, patient compliance may be affected by adverse reactions during treatment. Last but not least, in accordance with literature data, conventional immunotherapy would yield interesting desensitization figures but, on the contrary, the tolerance rates would be very discrete. As a consequence, the guidelines for the treatment of FA do not still recommend this kind of therapy.

In order to solve these drawbacks, the use of adjuvants to facilitate the induction of “protective” tolerogenic immune responses may be an interesting approach. Thus, for sublingual and oral immunotherapies, nanoparticles may act as mucosal adjuvants due to their inherent capability to carry the loaded allergen towards the surface of the epithelium and, then, facilitate its uptake and presentation by the APCs. In addition, some kinds of nanoparticles, such as those based on the use of the copolymer of methyl vinyl ether and maleic anhydride would induce innate immune responses (mediated by TLR-2 and TLR-4 as well as maturation of DCs and a biased Th1 response). In any case, nanoparticles may be used to co-encapsulate the allergen with an immunopotentiator (e.g. CpG motifs, LPS). All of these strategies are devoted to promote an intense and lasting “protective” Th1 response whilst avoiding systemic side

effects associated with cross-linking of IgE on mast cells and basophils, which implies the risk related to conventional whole allergen extracts. On the other hand, the use of nanoparticles may be also a good strategy to deliver orally plasmid DNA encoding for dominant allergens that may be transduced in the intestinal epithelium. This expression of the protein encoded by the pDNA would induce tolerance. Although further studies are necessary to really verify the potential of this approach in clinical management, all of these capabilities of nanoparticles open new possibilities for the treatment of FA.

Finally, during the last years, another interesting strategy to treat FA based on monoclonal antibodies has been proposed. In this case, the specific design of antibodies against circulating IgE or specific cytokines (IL-5) may block the generation of an allergic reaction. This approach shows an important advantage over allergen immunotherapies. Monoclonal antibodies are not designed to treat just one specific food allergy but, on the other hand, they provide neither a desensitization effect nor a tolerogenic status to the patient. However, one aspect to be explored would be the potential improvement on the immunotherapy effects associated with these monoclonal antibodies. In summary, more proactive treatment for food allergy is urgently needed to address the associated health risk and social burden.

Funding

This paper was not funded.

Declaration of interest

The research leading to these results has received funding from the European Community's Seventh Framework Programme [FP7/2007-2013] for ALEXANDER under grant agreement n° NMP-2011-1.2-2-28076. A Brotons-Canto acknowledges the "Asociación de Amigos Universidad de Navarra" for the financial support. The authors have no other 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 apart from those disclosed.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

- [1] Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. *Nutr Res* 2011;31:61-75
- [2] Mermelstein NH. Food allergies and other food sensitivities. *ASDC J Dent Child* 1986;53:296-9
- [3] Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. *Allergy* 2014;69:1008-25

- **Overview of the "European Academy of Allergy and Clinical Immunology" describing epidemiology, diagnosis, and acute management of non-life-threatening reactions.**

[4] Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol 2010;125: 116-25

- **A comprehensive review of food allergy and the different strategies available for its management**

[5] Chinthrajah RS, Hernandez JD, Boyd SD, Galli SJ, Nadeau KC. Molecular and cellular mechanisms of food allergy and food tolerance. J Allergy Clin Immunol 2016 4;137:984-97

[6] Gonzalez-Delgado P, Fernandez J, Caparros E, Moreno MV, et al. Clinical and immunological characteristics of a pediatric population with food protein-induced enterocolitis syndrome (FPIES) to fish. Pediatr Allergy Immunol 2016;27:269-75

[7] Jyonouchi H. Non-IgE mediated food allergy. Inflamm Allergy Drug Targets 2008;7:173-80

[8] Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. J Allergy Clin Immunol 2005;115:3-12

- **A comprehensive overview of oral tolerance and data related to food hypersensitivity wherever evidence is available.**

[9] Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 2014;3:141-53

[10] Jung C, Hugot JP, Barreau F. Peyer's patches: The immune sensors of the intestine. Int J Inflam 2010; 2010:823710

- [11] Pelaseyed T, Bergström JH, Gustafsson JK et al. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunol Rev* 2014;260:8-20
- [12] Feng T, Elson CO. Adaptive immunity in the host-microbiota dialog. *Mucosal Immunol* 2011;4:15-21
- [13] Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 2004;5:987-95
- [14] Menard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. *Mucosal Immunol* 2010;3:247-59
- [15] Miron N, Cristea V. Enterocytes: Active cells in tolerance to food and microbial antigens in the gut. *Clin Exp Immunol* 2012;167:405-12
- [16] Appleman LJ, Boussiotis VA. T cell anergy and costimulation. *Immunol Rev* 2003;192:161-80
- [17] Marth T, Zeitz Z, Ludviksson B, Strober W, Kelsall B. Murine model of oral tolerance. induction of fas-mediated apoptosis by blockade of interleukin-12. *Ann N Y Acad Sci* 1998;17;859:290-4
- [18] van Wijk F, Knippels L. Initiating mechanisms of food allergy: Oral tolerance versus allergic sensitization. *Biomed Pharmacother* 2007;61:8-20
- [19] Mondoulet L, Dioszeghy V, Vanoirbeek JAJ, Nemery B, Dupont C, Benhamou P. Epicutaneous immunotherapy using a new epicutaneous delivery system in mice sensitized to peanuts. *Int Arch Allergy Immunol* 2011;154:299-309
- [20] Untersmayr E, Jensen-Jarolim E. The role of protein digestibility and antacids on food allergy outcomes. *J Allergy Clin Immunol* 2008;121:1301-8

[21] Waserman S, Watson W. Food allergy. *Allergy Asthma Clin Immunol* 2011;7:S7

[22] Chafen JJ, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttorp MJ, et al. Diagnosing and managing common food allergies: a systematic review. *JAMA* 2010;303:1848-56

[23] Macchia D, Melioli G, Pravettoni V, Nucera E, Piantanida M, Caminati M, et al. Guidelines for the use and interpretation of diagnostic methods in adult food allergy. *Clin Mol Allergy* 2015;13:27

[24] de Silva D, Geromi M, Panesar SS, Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. Acute and long-term management of food allergy: systematic review. *Allergy* 2014;69:159-67

•Overview of the acute and long-term management of food allergy, including pharmacological and nonpharmacological interventions.

[25] Sampson HA. Food allergy. *J Allergy Clin Immunol* 2003;111:540-7

[26] O'Keefe AW, De Schryver S, Mill J, Mill C, Dery A, Ben-Shoshan M. Diagnosis and management of food allergies: new and emerging options: a systematic review. *J Asthma Allergy*. 2014;7:141-64

[27] Pereira B, Venter C, Grundy J, Clayton CB, Arshad SH, Dean T. Prevalence of sensitization to food allergens, reported adverse reaction to foods, food avoidance, and food hypersensitivity among teenagers. *J Allergy Clin Immunol* 2005;116:884-92

[28] Macdougall CF, Cant AJ, Colver AF. How dangerous is food allergy in childhood? the incidence of severe and fatal allergic reactions across the UK and Ireland. *Arch Dis Child* 2002;86:236-9

- [29] Grimshaw KE, Bryant T, Oliver EM, Martin J, et al. Incidence and risk factors for food hypersensitivity in UK infants: results from a birth cohort study," Clin. Transl. Allergy. 2015;6;1
- [30] Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C, et al. The prevalence of plant food allergies: A systematic review. J Allergy Clin Immunol 2008;121:1210-18
- [31] Bunyavanich S, Rifas-Shiman SL, Platts-Mills TA, Workman L, Sordillo JE, Gillman MW, et al. Peanut allergy prevalence among school-age children in a US cohort not selected for any disease. J Allergy Clin Immunol 2014;134:753-5
- [32] Ben-Shoshan M, Harrington DW, Soller L, Fragapane J, Joseph L, St Pierre Y, et al. A population-based study on peanut, tree nut, fish, shellfish, and sesame allergy prevalence in Canada. J Allergy Clin Immunol 2010;125:1327-35
- [33] Koplin JJ, Wake M, Dharmage SC, Matheson M, Tang ML, Gurrin LC, et al. Cohort profile: The HealthNuts study: Population prevalence and environmental/genetic predictors of food allergy. Int J Epidemiol 2015;44:1161-71
- [34] Osterballe M, Mortz CG, Hansen TK, Andersen KE, Bindslev-Jensen C. The prevalence of food hypersensitivity in young adults. Pediatr Allergy Immunol 2009;20:686-92
- [35] Shek LP, Cabrera-Morales EA, Soh SE, Gerez I, Ng PZ, Yi FC, et al. A population-based questionnaire survey on the prevalence of peanut, tree nut, and shellfish allergy in 2 asian populations. J Allergy Clin Immunol 2010;126:324-31
- [36] Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, et al. National prevalence and risk factors for food allergy and relationship to asthma: Results from the

national health and nutrition examination survey 2005-2006. *J Allergy Clin Immunol* 2010;126:798-806

[37] Ben-Shoshan M, Kagan RS, Alizadehfar R, Joseph L, Turnbull E, St Pierre Y, et al. Is the prevalence of peanut allergy increasing? A 5-year follow-up study in children in Montreal. *J Allergy Clin Immunol* 2009;123:783-8

[38] Luccioli S, Ross M, Labiner-Wolfe J, Fein SB. Maternally reported food allergies and other food-related health problems in infants: characteristics and associated factors. *Pediatrics* 2008;122:105-12

[39] Falcao H, Lunet N, Lopes C, Barros H. Food hypersensitivity in portuguese adults. *Eur J Clin Nutr* 2004;58:1621-5

[40] Katz Y, Rajuan N, Goldberg MR, Eisenberg E, Heyman E, Cohen A, et al. Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. *J Allergy Clin Immunol* 2010;126:77-82

[41] Venter C, Pereira B, Voigt K, Grundy J, Clayton CB, Higgins B, et al. Prevalence and cumulative incidence of food hypersensitivity in the first 3 years of life. *Allergy* 2008;63:354-9

[42] Schoemaker AA, Sprickelman AB, Grimshaw KE, Roberts G, Grabenhenrich L, Rosenfeld L, et al. Incidence and natural history of challenge-proven cow's milk allergy in european children--EuroPrevall birth cohort. *Allergy* 2015;70:963-72

[43] Xepapadaki P, Fiocchi A, Grabenhenrich L, Roberts G, Grimshaw KE, Fiandor A, et al. Incidence and natural history of hen's egg allergy in the first 2 years of life-the EuroPrevall birth cohort study. *Allergy* 2016;71:350-7

[44] Patel DA, Holdford DA, Edwards E, Carroll NV. Estimating the economic burden of food-induced allergic reactions and anaphylaxis in the United States. *J Allergy Clin Immunol* 2011;128:110-115

[45] Fox M, Mugford M, Voordouw J, Cornelisse-Vermaat J, Antonides G, de la Hoz CB, et al. Health sector costs of self-reported food allergy in Europe: A patient-based cost of illness study. *Eur J Public Health* 2013;23:757-62

[46] Gupta R, Holdford D, Bilaver L, Dyer A, Holl JL, Meltzer D. The economic impact of childhood food allergy in the United States. *JAMA Pediatr* 2013;167:1026-31

[47] Robinson MN, Dharmage SC, Tang ML. Comparison of adrenaline auto-injector devices: ease of use and ability to recall use. *Pediatr Allergy Immunol* 2014;25:462-7

[48] Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol* 2011;127:51-55

[49] Steinke JW, Lawrence MG. T-cell biology in immunotherapy. *Ann Allergy Asthma Immunol* 2014;112:195-9

[50] Gamazo C, Gastaminza G, Ferrer M, Sanz ML, Irache JM. Nanoparticle based-immunotherapy against allergy. *Immunotherapy* 2014;6:885-97

• **Review focused in allergen immunotherapy using nanoparticles as allergen delivery system**

[51] Bousquet J. EAACI position paper. *Allergy* 2000;55:116-34

[52] Straley DR. Office IgE-mediated environmental allergy evaluation and treatment. *Osteopathic Family Physician* 2013;5:9-16

[53] Mousallem T, Burks AW. Immunology in the clinic review series; focus on allergies: Immunotherapy for food allergy. *Clin Exp Immunol* 2012;167:26-31

[54] Wang J, Sampson HA. Oral and sublingual immunotherapy for food allergy. *Asian Pac J Allergy Immunol* 2013;31:198-209

• **Comprehensive comparison of oral and sublingual immunotherapy for food allergy**

[55] Di Rienzo V, Marcucci F, Puccinelli P, Parmiani S, Frati F, Sensi L, et al. Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: A 10-year prospective study. *Clin Exp Allergy* 2003;33:206-10

[56] Allam JP, Stojanovski G, Friedrichs N, Peng W, Bieber T, Wenzel J, et al. Distribution of Langerhans cells and mast cells within the human oral mucosa: New application sites of allergens in sublingual immunotherapy? *Allergy* 2008;63:720-7

[57] Fleischer DM, Burks AW, Vickery BP, Scurlock AM, Wood RA, Jones SM, et al. Sublingual immunotherapy for peanut allergy: A randomized, double-blind, placebo-controlled multicenter trial. *J Allergy Clin Immunol* 2013;131:119-27

[58] Jones SM, Burks AW, Dupont C. State of the art on food allergen immunotherapy: Oral, sublingual, and epicutaneous. *J Allergy Clin Immunol* 2014 2;133:318-23

•• **Systemic review of the actual stage of clinical investigations in the 3 major forms of specific immunotherapy: oral, sublingual and epicutaneous.**

[59] Kim EH, Bird JA, Kulis M, Laubach S, Pons L, Shreffler W, et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol* 2011;127:640-6

[60] Anagnostou K, Clark A. Peanut immunotherapy. *Clin Transl Allergy* 2014;4:7022-30

[61] Enrique E, Pineda F, Malek T, Bartra J, Basagana M, Tella R, et al. Sublingual immunotherapy for hazelnut food allergy: a randomized, double-blind, placebo-

controlled study with a standardized hazelnut extract. *J Allergy Clin Immunol* 2005;116:1073-9

[62] Fernandez-Rivas M, Garrido Fernandez S, Nadal JA, Diaz de Durana MD, Garcia BE, Gonzalez-Mancebo E, et al. Randomized double-blind, placebo-controlled trial of sublingual immunotherapy with a prup 3 quantified peach extract. *Allergy* 2009;64:876-83

[63] Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009;124:292-300

[64] Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. *Allergy* 2007;62:1261-9

[65] Martorell Calatayud C, Muriel Garcia A, Martorell Aragonés A, De La Hoz Caballer B. Safety and efficacy profile and immunological changes associated with oral immunotherapy for IgE-mediated cow's milk allergy in children: systematic review and meta-analysis. *J Investig Allergol Clin Immunol* 2014;24:298-307

[66] Caminiti L, Pajno GB, Crisafulli G, Chiera F, Collura M, Panasci G, et al. Oral immunotherapy for egg allergy: a double-blind placebo-controlled study, with post-desensitization follow-up. *J Allergy Clin Immunol Pract* 2015;3:532-9

[67] Buchanan AD, Green TD, Jones SM, Scurlock AM, et al. Egg oral immunotherapy in nonanaphylactic children with egg allergy. *J Allergy Clin Immunol* 2007;119:199-205

[68] Vickery BP, Scurlock AM, Jones SM, Burks AW. Mechanisms of immune tolerance relevant to food allergy. *J Allergy Clin Immunol* 2011;127:576-84

- [69] Syed A, Garcia MA, Lyu SC, Bucayu R, Kohli A, Ishida S, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol* 2014;133:500-10
- [70] Praticò A,D., Leonardi S. Immunotherapy for food allergies: A myth or a reality? *Immunotherapy* 2015;7:147-61
- [71] Mondoulet L, Dioszeghy V, Thebault C, Benhamou PH, Dupont C. Epicutaneous immunotherapy for food allergy as a novel pathway for oral tolerance induction. *Immunotherapy* 2015;7:1293-305
- [72] Dupont C, Kalach N, Soulaines P, Legoue-Morillon S, Piloquet H, Benhamou PH. Cow's milk epicutaneous immunotherapy in children: a pilot trial of safety, acceptability, and impact on allergic reactivity. *J Allergy Clin Immunol* 2010;125:1165-7
- [73] Dupont C, Bourrier T, de Blay F, Guénard-Bilbault L, Sauvage C, Cousin M, et al. Peanut epicutaneous immunotherapy (EPIT) in peanut-allergic children: 18 months treatment in the arachild study. *J Allergy Clin Immunol* 2014;133:AB102
- [74] Mondoulet L, Dioszeghy V, Puteaux E, Ligouis M, Dhelft V, Letourneur F, et al. Intact skin and not stripped skin is crucial for the safety and efficacy of peanut epicutaneous immunotherapy (EPIT) in mice. *Clin Transl Allergy* 2012;2:22
- [75] Li X, Srivastava K, Grishin A, Huang C, Schofield B, Burks W, et al. Persistent protective effect of heat-killed *Escherichia coli* producing “engineered” recombinant peanut proteins in a murine model of peanut allergy. *J Allergy Clin Immunol* 2003;112:159-67

[76] Swoboda I, Bugajska-Schretter A, Linhart B, Verdino P, Keller W, Schulmeister U, et al. A recombinant hypoallergenic parvalbumin mutant for immunotherapy of IgE-mediated fish allergy. *J Immunol* 2007;178:6290-6

[77] Ma Y, Gadermaier G, Bohle B, Bolhaar S, Knulst A, Markovic-Housley Z, et al. Mutational analysis of amino acid positions crucial for IgE-binding epitopes of the major apple (*Malus domestica*) allergen, mal d 1. *Int Arch Allergy Immunol* 2006;139:53-62

[78] Francis JN, Durham SR. Adjuvants for allergen immunotherapy: experimental results and clinical perspectives. *Curr Opin Allergy Clin Immunol* 2004;4:543-8

• **This article covers the recent advances in development of new adjuvant and their formulation.**

[79] Pecquet S, Leo E, Fritsche R, Pfeifer A, Couvreur P, Fattal E. Oral tolerance elicited in mice by β -lactoglobulin entrapped in biodegradable microspheres. *Vaccine* 2000;18:1196-202

[80] Srivastava KD, Siefert A, Fahmy TM, Caplan MJ, Li XM, Sampson HA. Investigation of peanut oral immunotherapy with CpG/peanut nanoparticles in a murine model of peanut allergy. *J Allergy Clin Immunol* 2016;138:536-43

[81] Bohle B, Jahn-Schmid B, Maurer D, Kraft D, Ebner C. Oligodeoxynucleotides containing CpG motifs induce IL-12, IL-18 and IFN- γ production in cells from allergic individuals and inhibit IgE synthesis in vitro. *Eur J Immunol* 1999;29:2344-53

[82] Tamayo I, Irache JM, Mansilla C, Ochoa-Reparaz J, Lasarte JJ, Gamazo C. Poly(anhydride) nanoparticles act as active Th1 adjuvants through Toll-like receptor exploitation. *Clin Vaccine Immunol* 2010;17:1356-62

- [83] Denzel A, Maus UA, Rodriguez Gomez M, Moll C, Niedermeier M, Winter C, et al. Basophils enhance immunological memory responses. *Nat Immunol* 2008;9:733-42
- [84] Gomez S, Gamazo C, San Roman B, Ferrer M, Sanz ML, Irache JM, Gantrez AN nanoparticles as an adjuvant for oral immunotherapy with allergens. *Vaccine* 2007;25:5263-71
- [85] Gomez S, Gamazo C, San Roman B, Ferrer M, Sanz ML, Espuelas S, et al. Allergen immunotherapy with nanoparticles containing lipopolysaccharide from *Brucella ovis*. *Eur J Pharm Biopharm* 2008;70:711-7
- [86] De Souza Reboucas J, Irache JM, Camacho AI, Gastaminza G, Sanz ML, Ferrer M, et al. Immunogenicity of peanut proteins containing poly(anhydride) nanoparticles. *Clin Vaccine Immunol* 2014;21:1106-12
- [87] Carroll EC, Jin L, Mori A, Munoz-Wolf N, Oleszycka E, Moran HB, et al. The vaccine adjuvant chitosan promotes cellular immunity via DNA sensor cGAS-STING-dependent induction of type I interferons. *Immunity* 2016;44:597-608
- [88] Roy K, Mao HQ, Huang SK, Leong KW. Oral gene delivery with chitosan--DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nat Med* 1999;5:387-91
- [89] Wang J, Patil SP, Yang N, Ko J, Lee J, Noone S, et al. Safety, tolerability, and immunologic effects of a food allergy herbal formula in food allergic individuals: a randomized, double-blinded, placebo-controlled, dose escalation, phase 1 study. *Ann Allergy Asthma Immunol* 2010;105:75-84
- [90] Niers LE, Timmerman HM, Rijkers GT et al Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clin Exp Allergy* 2005;35:1481-9

- [91] Flohr C, Quinnell RJ, Britton J. Do helminth parasites protect against atopy and allergic disease? *Clin Exp Allergy* 2009 ;39:20-32
- [92] Rail A, Do LAT, Katz R, Sheinkopf LE, Simons CW, Klaustermeyer W. Effects of omalizumab in patients with food allergy. *Allergy Asthma Proc* 2010;31:76-83
- [93] Umetsu DT, Rachid R, Schneider LC. Oral immunotherapy and anti-IgE antibody treatment for food allergy. *World Allergy Organ J* 2015;8:1-5
- [94] Lieberman JA, Chehade M. Use of omalizumab in the treatment of food allergy and anaphylaxis. *Curr Allergy Asthma Rep* 2013;13:78-84
- [95] Leung DY, Sampson HA, Yunginger JW, Burks AW, Jr, Schneider LC, Wortel CH, et al. Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med* 2003;348:986-93
- [96] Kraneveld AD, Sagar S, Garssen J, Folkerts G. The two faces of mast cells in food allergy and allergic asthma: The possible concept of Yin Yang. *Biochim Biophys Acta* 2012;1822:93-9
- [97] Busse WW, Ring J, Huss-Marp J, Kahn J. A review of treatment with mepolizumab, an anti-IL-5 mAb, in hypereosinophilic syndromes and asthma. *J Allergy Clin Immunol* 2010;125:803-13
- [98] Poole JA, Meng J, Reff M, Spellman MC, Rosenwasser LJ. Anti-CD23 monoclonal antibody, lumiliximab, inhibited allergen-induced responses in antigen-presenting cells and T cells from atopic subjects. *J Allergy Clin Immunol* 2005;116:780-8
- [99] Abonia JP, Putnam PE. Mepolizumab in eosinophilic disorders. *Expert Rev Clin Immunol* 2011;7:411-7

[100] Manohar M, Nadeau KC. The potential of anti-IgE in food allergy therapy. *Curr Treat Options Allergy* 2014;1:145-56

• **A comprehensive overview of anti-IgE used either as monotherapy or as adjuvant therapy for the treatment of food allergy**

[101] Schiavi E, Barletta B, Butteroni C, Corinti S et al. Oral therapeutic administration of a probiotic mixture suppresses established Th2 responses and systemic anaphylaxis in a murine model of food allergy. *Allergy* 2011;66:499-508

[102] Kim JY, Choi YO, Ji GE. Effect of oral probiotics (*Bifidobacterium lactis* AD011 and *Lactobacillus acidophilus* AD031) administration on ovalbumin-induced food allergy mouse model. *J Microbiol Biotechnol* 2008;18:1393-400

[103] Sistek D, Kelly R, Wickens K, Stanley T, Fitzharris P, Crane J. Is the effect of probiotics on atopic dermatitis confined to food sensitized children? *Clin Exp Allergy* 2006;36:629-33

[104] Tang MLK, Ponsonby A, Orsini F, Tey D, Robinson M, Su EL, et al. Administration of a probiotic with peanut oral immunotherapy: A randomized trial. *J Allergy Clin Immunol* 2015;135:737-44

Table 1. Estimated prevalence for peanut allergy in different countries. OFC: oral food challenge; SR: self-report; SPT: skin prick test; sIgE: food-specific IgE determinations; DBPCFC: double-blind, placebo-controlled food challenges; MA: meta-analysis.

Area	Age (years)	Sample size	Method	Prevalence	Ref
USA	7-10	616	SR, sIgE	2%	[31]
Canada	8-9	5161	SR, SPT, sIgE DBPCFC	1.6%	[32]
Australia	1	5276	SR, SPT, OFC	3%	[33]
Denmark	12-14	237	SR, DBPCFC	5.3% SR	[34]
			OFC, DBPCFC	0.6% OFC	[34]
Singapore	4-6	4390	SR	3.6%	[35]
Singapore	14-16	6450	SR	1.2%	[35]
Philippines	14-16	11322	SR	1.3%	[35]

Table 2. Estimated prevalence for shellfish allergy in different countries. OFC: oral food challenge; SR: self-report; Q: questionnaire; SPT: skin prick test; sIgE: food-specific IgE determinations; DBPCFC: double-blind, placebo-controlled food challenges; MA: meta-analysis.

Area	Age (years)	Sample size	Method	Prevalence	Ref
USA	General	8203	sIgE	1.0	[36]
Canada	General	9667	SR (perceived), SR+ Q (Probable); SPT/sIgE (Confirmed)	Perceived: 1.6% Probable: 1.4% Confirmed: 0.7%	[37]
	Children		SR (perceived), SR+ Q (Probable); SPT/sIgE (Confirmed)	Perceived: 0.6% Probable: 0.5% Confirmed: 0.1%	
	Adult		SR (perceived), SR+ Q (Probable); SPT/sIgE (Confirmed)	Perceived: 1.9% Probable: 1.7% Confirmed: 0.7%	
Singapore	4-6	4115	SR	1.2%	[35]
Singapore	14-16	6342	SR	5.2%	[35]
Philippines	14-16	11158	SR	5.1%	[35]

Table 3. Estimated prevalence for milk and egg allergy in different world regions. OFC: oral food challenge; SR: self-report; SPT: skin prick test; sIgE: food-specific IgE determinations; DBPCFC: double-blind, placebo-controlled food challenges; MA: meta-analysis.

Area	Age (years)	Sample size	Method	Prevalence	Ref
MILK					
USA	General	8203	SR, sIgE	0.4%	[36]
USA	1	9667	SR	0.4%	[38]
Portugal	> 39	659	SR	3.8%	[39]
Israel	1	13019	SR, SPT, OFC	0.5%	[40]
UK	3	642	SPT, OFC	0.5%	[41]
Europe	2	9336	SR, SPT, sIgE, DBPCFC	0.5%	[42]
EGG					
USA	General	8203	SR, sIgE	0.2%	[36]
Portugal	>39	659	SR	7.7%	[39]
UK	3	642	SPT, OFC	1.4%	[41]
Australia	1	5276	SR, SPT, OFC	9%	[33]
Europe	2	9336	SR, SPT, sIgE, DBPCFC	0.8%	[43]

Table 4. Monoclonal antibodies with potential application in food allergy.

Monoclonal antibody	Company	Activity	Application	Ref
Lumiliximab	Biogen Idec Limited	Anti-CD23	Binds to CD23 that regulates IgE production and allergy-induced immune and inflammatory responses, reducing Th2-type immune responses	[98]
Mepolizumab (Nucala®)	GSK	Anti-IL5	Binds to human interleukin 5 (IL-5), the key Th2 cytokine responsible for regulation of blood and tissue eosinophils	[99]
Omalizumab (Xolair®)	Genentec	Anti-IgE	Binds to circulating IgE, reducing IgE receptor expression, and decreasing mediator release from mast cells and basophils	[94]
TNX-901/ Talizumab	Tanox Genentec Novartis	Anti-IgE	Binds to circulating IgE and specifically target IgE-expressing B lymphocytes, without triggering the activation of mast cells and basophils	[100]

Figure 1. Schematic representation of the “gastrointestinal mucosal barrier”. Depicted are the pathways used for food allergens to reach and penetrate the mucosal: through DCs able to project “dendrites”, through specialized M cells or by transcellular and paracellular routes. In some circumstances, dietary proteins can be also taken up by intestinal epithelial cells.

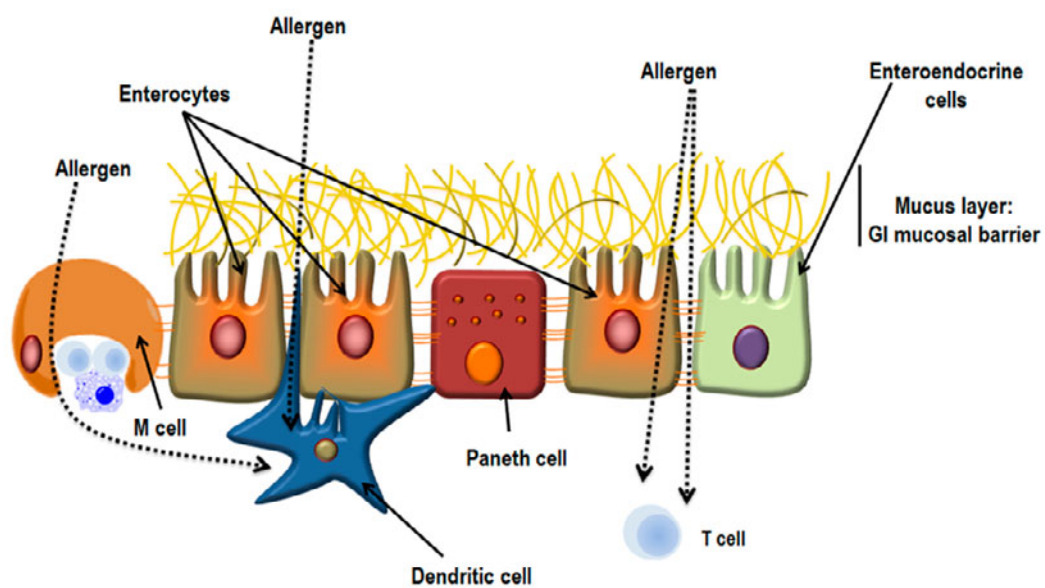


Figure 2. Mechanisms of allergic reactions. After an initial exposure, allergens are taken up by professional APCs that present peptides on MHC II ($CD80^+$) to naive T cells ($CD4^+$), which activate a cellular immune response and IgE production. Thereafter, high amounts of specific IgE antibodies are produced and bind to high affinity IgE receptor ($Fc\epsilon RI$) in membranes of circulating basophils and mast cells. This signal stimulates the release of proinflammatory substances such as histamine, serine proteases, platelet activating factor, cytokines, leukotrienes and prostaglandins, which are responsible for the symptoms and signs of allergic diseases.

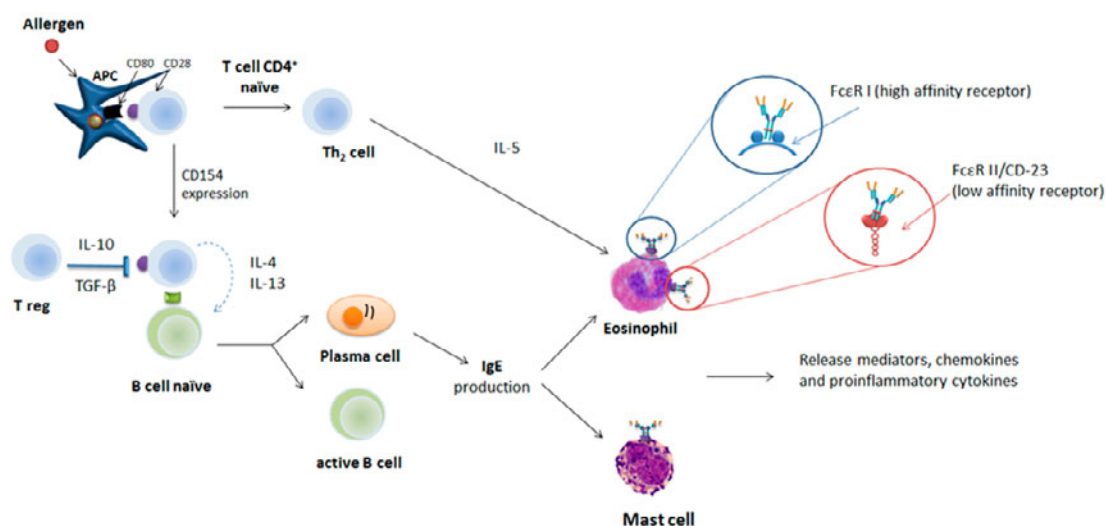


Figure 3. Effect of monoclonal antibodies in the reaction induced by a food allergen.

