

Review Article

New Insights in Immunotherapies for Food Allergies. A Path between the Mouse Model and the Allergic Patient

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Abstract

Allergic diseases are the most prevalent immunopathologies worldwide. Nowadays, allergen avoidance is the unique effective treatment for allergic patients. Pre-clinical studies and clinical trials have been successful to prove that immunotherapies may accomplish mucosal mechanisms of allergen-specific tolerance, which are able to revoke the allergic sensitization.

Although more than 100 years have elapsed since the first reported procedure achieved in patients sensitive to pollen, the main obstacle in these therapies still remains adverse reactions induced during treatment. The need for further studies is required to explore safe and effective therapeutic protocols. More recently, immunotherapy appears to be an attractive option for patients with food allergy, although it is still experimental. Different strategies were proposed to overcome the concerning adverse effects that compromise safety, effectiveness and compliance with treatments. At this point, translational medicine is a flourish field in the arenas of basic science, applied science, and clinical research. The use of experimental animals may provide new insights to unravel mechanisms that play key roles in desensitization and tolerance induction, and to modify existing protocols or design new therapeutic approaches. The present reviews describes the different immunotherapies that are used in allergic patients, the promising immune interventions that are currently evaluated in clinical trials and the contributions that animal models may provide to improve the quality of treatments.

Introduction

Allergic diseases are a global health concerning and result from a complex interaction between environmental factors and genes. Food allergy is an immune-mediated adverse reaction against antigenic foods and constitutes a growing clinical problem. As incidence of allergy escalated during the last decades in some highly industrialized regions, the prevalence of food allergy has raised at the same time mainly in infancy [1,2] and is becoming a growing issue in adults [3].

Despite the great progress made to elucidate the molecular basis of allergic disorders, it is not completely understood why some individuals develop allergic sensitization to some foods, while the majority of individuals are immunologically tolerant. In this regard evidence suggests that environmental factors are key inducers [4]. Although any food is potentially capable of causing an allergic reaction, a group of eight major allergenic foods, referred to as the “Big 8”, account for 90 % of all food allergic reactions in The United States. These are: peanut, tree nuts, cow’s milk, egg, soybean, wheat, fish and shellfish and its derivatives. Recent evidences may explain why certain foods are inherently allergenic [5,6].

A variety of symptoms involving the skin, gastrointestinal and respiratory tracts can be evidenced in allergic patients upon exposure to the offending food; these symptoms can be attributed to IgE-mediated and/or non-IgE mediated mechanisms. Genetic predisposition, environmental factors, route of exposure and maturity of the mucosal immune system may play a role in sensitization rather than oral tolerance to foods, leading to a Th2-biased food specific T

cell-mediated immune response underlying the detrimental reaction to the innocuous food.

Diagnosis of food allergy is complicated. It is a heterogeneous disorder involving several clinical entities with no single immunologic mechanism involved. Once food allergy is suspected the first clinical or even familial indication is avoidance of the suspicious food. Currently, there is no accepted form of therapy for food allergies. Patient and family should be educated to avoid exposure to the responsible allergen, and they should have immediate access to epinephrine in case of anaphylaxis when accidental ingestion occurred. In addition, there are no therapies proven to accelerate the development of oral tolerance or provide effective protection from accidental exposures. However, novel allergen specific and allergen non-specific approaches to food allergy therapy are under study [7].

Immunotherapy is nowadays a treatment procedure to induce, enhance or suppress an immune response in different immunological disorders (cancer, autoimmunity, allergy, etc). Although allergen specific immunotherapy has been used for the treatment of IgE-mediated allergy longer than a hundred years, the first randomized clinical trial of oral immunotherapy for food allergy was done in 2008. Of note, this therapeutic procedure has not been accepted as a routine treatment for food allergy.

The aim of food allergy immunotherapy is to step-wise induce desensitization followed with a permanent restoration of oral tolerance. The term *desensitization* accounts for a temporary hypo-responsiveness during regular ingestion of the food, as dosing is

discontinued, the protective effect is lost. *Tolerance* is defined as a prolonged ability to ingest large amounts of food proteins with no detrimental reaction, being immunotherapy completed. Different reports describe advances made in oral, sublingual and subcutaneous food immunotherapies, with some approaches and innovations derived from animal model studies [8].

In this sense, mouse models of allergic diseases provide an essential tool for studying the pathogenesis of allergic diseases, and the development of novel or modified therapeutic strategies. As mice do not develop allergy spontaneously, it is induced artificially through the use of pro-Th2 adjuvants (aluminum hydroxide, cholera toxin, Staphylococcus enterotoxin B, etc.). The artificial sensitization method employed limited the use of these experimental animals to study the inductive phase of food allergy. During the sensitization phase, the repeated intragastric administration of food proteins in combination with a potent mucosal adjuvant, cholera toxin, abrogates oral tolerance to the co-administered antigens, and establishes the disease state, which can be easily evidenced with the clinical signs elicited minutes after the exposure to the food allergen [9,10]. A variety of *in vitro* and *in vivo* parameters can be evaluated to characterize the allergic status of sensitized mice [10-12]. These experimental models have been successfully employed to explore the effects of different immunotherapeutic strategies on the immune system. The ability to sensitize animals to a specific food through the intragastric administration of allergens, and then elicit an allergic response by activating mucosal immune cells provides clear ties to the human condition. In addition, mouse models constitute useful biological tools to study novel mucosal adjuvants, both to induce a Th2 immunoregulatory immune response for Th1-mediated immunopathologies (autoimmunity), and to modulate it (allergic disorders).

Oral immunotherapy

Oral immunotherapy (OIT) consists in a daily consumption of milligrams to grams of the selected allergen, which is incrementally raised over weeks to months with the goal of inducing desensitization and then tolerance [13-15]. In the first randomized double-blind placebo-controlled OIT trial performed by Skripak et al., 20 children with IgE-mediated cow's milk allergy were randomized given milk or placebo. The main point of this study was that patients tolerated 128 times higher amounts of milk compare with patients before treatment or with placebo-treated patients [15]. Despite no variation on serum specific IgE levels, IgG4 and titration SPT threshold were increased, and most of the patients experienced transient adverse reactions after OIT treatment. Therefore, this study did not provide a clear evidence of tolerance induction. Keet et al. showed that 40 % of subjects receiving milk OIT passed an oral food challenge when treatment was ceased for 6 weeks, however some regained reactivity within a week [16]. Similar results were observed in OIT with egg and peanut allergens [17,18]. Although data on long-term treatment are limited, Keet et al. showed that, three to five years after cow's milk OIT was finished, only 25% of subjects were consuming normal amounts of milk without any symptoms, and that almost 20% of treated patients experienced anaphylactic reactions during the follow-up period, including children who appeared to have a good response to treatment [19].

Additionally, peanut allergy is the most common cause of fatal food allergic reactions [20] since peanut components are widely used in processed foods, and some of them were probed to be highly immunogenic [21]. This means that fatal or nearly fatal reactions to this life-threatening allergen are responsible for several deaths in the United States (thousands of hospitalizations and hundreds of fatal cases per year) following accidental exposure to this allergen [22,23]. Although, there are no immunotherapy regimens in routine use for peanut allergy, peanut OIT has showed promising results. Most peanut OIT protocols involve an initial escalation phase (days) of orally administered peanut. This is followed by administration of further build-up doses (months), and then maintenance doses (months). The maximum maintenance doses are between 300 mg and 4000 mg of peanut protein. While some studies have shown hopeful results, the risk of severe reactions during allergen administration is of concern [24,25]. Anagnostou et al. have investigated the role of peanut OIT in 99 children with peanut allergy in a phase II, randomized-controlled trial. Subjects who successfully completed the OIT protocol had a 25-fold increase of their peanut threshold [26].

Despite the number of promising OIT studies and the increasing interest of the medical community in the development of a routine OIT using native proteins, a high percentage of patients still suffer *adverse side effects*. One of the therapeutic strategies assessed to overcome it was to include modified allergenic proteins (baked or roasted) in the sequential steps of allergen administration. The addition of baked milk or egg to the diet of allergic children that tolerated such baked foods accelerated the development of tolerance to unbaked milk or egg compared with patients that did not tolerate the processed food and received a free-allergen diet [27,28]. It was observed that this procedure induced mild adverse reactions, which were easily controlled.

In summary, OIT has showed promising results in cow's milk, peanut and egg white allergen patients, with the primarily induction of desensitization and further tolerance. However, the high rate of adverse reactions elicited during the immunotherapy, and the uncertainty of long term outcome require further studies. Furthermore, the immunological mechanisms underlying desensitization and tolerance mechanisms in OIT have not been fully investigated (Table 1). In this sense, experimental animals may provide relevant information in a way that would be nonviable in clinical trials.

A relatively new form of non-specific immunotherapy is the

Table 1: Mechanisms induced in antigen-specific immunotherapies.

Immunotherapy	Study	Antigen	Mechanism	References
OIT	Human	Peanut Milk Egg	Treg, IL-10, TFG-β	[71-73]
	Mouse	Milk Egg Peanut	Treg, IL-10, TFG-β	[37,74,75]
SLIT	Human	Milk Peanut	IL-10, Th1, IgG4	[27,76,77]
	Mouse	--	--	No ref.
SCIT	Human	Milk Peanut	Treg, IL-10, increase IgA, Igg4	[62,63]
	Mouse	--	--	No ref.

anti-IgE therapy (with humanized α -IgE monoclonal antibody-Omalizumab), which has been successfully used in asthma. Sampson et al. started a phase II clinical trial with Omalizumab in 150 patients with peanut allergy; however the study was terminated early due to the severe reactions that occurred during the peanut challenge [29]. Recently, a combination therapy was studied using the α -IgE antibody in patients undergoing OIT. It was observed that the biological was effective in reducing adverse reactions during the oral administration of the allergen (cow's milk, wheat, peanut and egg), with a rapid oral desensitization [30-32]. Although these clinical trials were effective for mono-allergic and multi-allergic patients, reaching daily doses of 4000 mg for individual allergens during several weeks, tolerance was not demonstrated [33]. However, several potential benefits can be speculated with the combination of both therapies. An additional strategy to reduce the risk of adverse reactions is the use of modified allergens. The efficacy of this therapy was evaluated in a humanized mouse model [34]. OIT with dietary food produce a high rate of adverse side effects, due to the intact B cell epitopes in native allergenic proteins with capacity to cross-link IgE bound to mast cell and basophil membrane receptors. The use of peptides containing T epitopes has been reported to be a safe and effective option for OIT in animal models of asthma to mite allergen [35,36] and food allergy to egg [37]. Finally, food allergy mouse models were used to test mutated allergens, in which immunodominant B cell epitopes were modified by point mutation to impair allergenicity, and to develop a safer immunotherapy [38-40].

Sublingual immunotherapy

Sublingual immunotherapy (SLIT) involves frequent placement of micrograms to milligrams of allergen under the tongue. Cochrane analyses have confirmed the efficacy and safety of sublingual therapy for allergic rhinitis with long-term benefits (1-2 years) [41]. Although most of the SLIT studies have focused on inhalant allergies, emerging clinical trials with SLIT have shown promising results at inducing desensitization in food allergy [42,43]. However, SLIT is not currently recommended for treatment of food allergy [44]. Only few studies have been done in peanut, nuts, kiwi, peach and cow's milk allergy [45-49] and they showed lower frequencies of adverse reactions compared with OIT, which is likely due to lower doses of allergen administration and the capacity of the mouth mucosa to induce intestinal tolerance. However, the efficacy of SLIT is still debated. Considering that the induction of Treg is the key mechanism underlying desensitization and tolerance, it has been reported that the current protocols of SLIT have a lower efficiency as compared to OIT (Table 1) [50,51]. Nevertheless, SLIT protocols used for egg, cow's milk or peanut allergy [52] showed a significant increase in the threshold dose of the food allergen to induce allergic symptoms, a decrease in serum specific IgE along with a rise in serum IgG4 levels, and induction of Tregs [40]. There are only two randomized studies comparing oral and sublingual immunotherapies. Keet et al. conducted a clinical trial with 30 milk allergic patients comparing SLIT alone versus SLIT followed by OIT. It was reported that despite the similar number of total adverse food reactions between groups, the number of systemic reaction was higher in the OIT groups. Of note, the oral challenge performed after 60 weeks of maintenance doses, rendered a higher allergen threshold in SLIT followed by OIT treated patients compared with SLIT-treated patients [16]. Narisety

et al. performed a similar randomized study with peanut allergic patients to compare the safety and efficacy of OIT and SLIT. They found a partial desensitization with at least a 10-fold increases in peanut challenge threshold compared with baseline. They similarly found that efficacy of OIT was greater than SLIT, although safety is still greater in patients receiving SLIT compared with OIT [18].

The literature shows scarce reports exploring SLIT in mouse models. Most of them employed mucosal adjuvants and were done in rhinitis and asthma. Lombardi et al. showed that Pam3CSK4, a known TLR2 agonist, exhibits immunomodulatory properties with the induction of CD4⁺ T cells that secreted IL-10 and IFN- γ to control the allergic reaction [53]. Saint Lu et al. used a mucoadhesive form of chitosan to enhance allergen uptake, processing and presentation by dendritic cells. It was shown that the treatment enhances tolerance induction in mice with established asthma, with a reduction of lung inflammation [54]. Of note, there is no study with experimental animals using SLIT to show that immunomodulatory mechanisms can be induced in the sublingual mucosa, with the subsequent control of the intestinal inflammation and the systemic allergy. Based on our preliminary data showing that the sublingual administration of the allergen, or the allergen in combination with Th1 adjuvants, can control hypersensitivity symptoms upon allergen oral challenge, we consider that food allergy mouse models are indeed a valuable biological tool to study pre-clinical strategies for food allergy immunotherapy. Using a cholera toxin-driven IgE-mediated milk allergy model, we could revert an established Th2-biased intestinal immune response with the oral administration of milk proteins, and the subsequent induction of Tregs (data not published).

In summary, SLIT in humans has been clearly associated with a substantially reduced risk of adverse reactions, although efficacy and long-term effectiveness are the main drawbacks. Food allergy mouse models may provide information for new hopes to improve this disease-modifying therapy.

Subcutaneous and epicutaneous immunotherapy

The major form of allergen-specific immunotherapy is the subcutaneous immunotherapy (SCIT). Subcutaneous injections of the allergen deliver the allergen to skin Langerhans cells, with the subsequent migration of dendritic cells to lymph nodes and further regulation of effector allergic responses [55]. Although, it is widely used for treatment of allergy, it is not accepted as a routine therapy, especially for children. Recruitment of patients and then (less than 5% of all allergic patients) compliance to SCIT (less than 25% of patients drop out within the first year of treatment) are difficult to achieve [56].

Safety and effectiveness of SCIT have been reported in rhinitis and asthma [57]. Although adverse reactions have been documented, the vast majority are mild and very rarely fatal [58]. In contrast, clinical trials of SCIT in peanut allergic patients showed that anaphylactic side-effects induced during the administration of the native allergen are highly frequent [59,60]. The first attempt to improve this protocol was done by The European multicenter consortium that conducts the prospective Food Allergy Specific Immunotherapy (FAST) project to evaluate the use of alum-adsorbed hypoallergens to control fish and peach allergy. Recombinant mutated allergens, with decreased IgE-binding capacity and intact T cell-reactivity capacity, are included in

the SCIT. Patients will be enrolled for a phase I/IIA initial clinical trial, while animal models will be used to evaluate the allergenicity of the recombinant hypoallergenic proteins developed and efficacy of the immunotherapy [61].

More recently, epicutaneous immunotherapy (EPIT) with allergen-embedded patch administration has been carried out in food allergy. One of the first clinical trials for EPIT evaluated 18 children with cow's milk allergy during three months. EPIT not showed serious systemic adverse effects. Although treatment was associated with more frequent complaints for local pruritus and discomfort than placebo, patient compliance was not compromised [62]. In addition, there is a phase I clinical trial that is conducted in the United States with 80 peanut allergic patients that received EPIT and 20 placebo-treated subjects; mild or moderate local adverse effects were experienced by 90% of patients [63]. Another clinical trial in phase IIA with 54 children with peanut allergy that underwent EPIT showed a good response. Oral food challenges conducted at 6-month intervals over a 18-month period revealed that 67 % of patients achieved desensitization, with a 10-fold increase in cumulative reactive dose from baseline [64,65].

Despite the scarce number of reports, studies of allergen patch in animal models indicate a potential application of this therapy. Pre-clinical studies in mice were carried out with peanut, ovalbumin and some aeroallergens using a skin patch [66-68]. EPIT on intact skin down-modulated the allergic immune response through the induction of long-lasting tolerogenic Treg [69]. The protection offered by EPIT-induced Tregs was adoptively transferred to peanut sensitized animals [68,70].

Remarkably, there is no study of SCIT in animal models, thus reflecting the difficulty to immunomodulate allergic disorders through this administration route in mice.

Conclusions

Immunotherapy appears to be a promising option for the treatment of food allergy. OIT and SLIT have emerged as attractive therapies to divert the aberrant immunological mechanisms underlying these diseases. Both modalities have shown to induce desensitization through different mechanisms (Table 1). Although it has been demonstrated that a sustained unresponsiveness can be achieved with OIT, several clinical trials support that safety, feasibility, compliance, and economic profile is mainly found with the sublingual immunotherapy. However, further research is needed to improve its efficacy and long-term effectiveness. In this scenario, mouse models represent a valuable tool for evaluating novel or modified forms of immunotherapy and also for dissecting the mechanism of action. The combination of specific methods or even both specific and non-specific methods may enhance desensitization with a rapid onset of tolerance.

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