



Immunotherapy for food allergies: a myth or a reality?

Food allergy is a worldwide issue, with an estimated prevalence of 2–10%. An effective treatment is not available for people affected and the only management is the avoidance of the allergen. Oral immunotherapy and sublingual immunotherapy have been tested by several authors, in particular for milk, egg and peanuts allergy, with significant results in term of desensitization induction. The achievement of tolerance is by the contrary doubtful, with different results obtained. In this review, we reviewed protocols of oral and sublingual immunotherapy for food allergy published in literature, mainly against milk, egg and peanut. At present, immunotherapy does not represent the gold standard in the treatment of food allergy, even if it can desensitize patients.

Keywords: allergy • desensitization • egg • food • immunotherapy • milk • peanuts • tolerance

Food allergy affect roughly 15 million Americans and 17 million Europeans, mostly children, and its prevalence has shown an important growth in the last decades, ranging from 2 to 10% of people [1,2]. There is also evidence that heritable factors influence the impact of hygiene-related exposures on the risk of having allergy. A number of important gene–environment interactions have been identified [3].

The necessity of a definitive treatment for this important issue has encouraged scientists and physicians to try new appropriate therapeutic strategies. Based on the positive results obtained in the last decades by immunotherapy for air allergens, a constantly growing number of protocols have been tested in several centers on the use of oral immunotherapy (OIT) or sublingual immunotherapy (SLIT). The first choice appears more effective but associated more frequently with severe systemic reactions, while the second appears to be safer but less effective [4].

Aside the occurrence of side effects, the debate on food immunotherapy is centered whether it can induce the development of

desensitization or tolerance. In the first case, the effect is transitory, reversible and has to be maintained with regular intakes of allergenic food, because its discontinuation leads to the return of previous clinical reactivity. In the case of the tolerance, a relatively long-lasting effect is obtained and the the suspension of the food does not lead to change in the immune status [1].

Immunotherapy for food allergies: oral versus sublingual

Several food allergy immunotherapy protocols have been tested in the last 25 years, but to date it is not clear if this therapy represents still a myth or a reality to resolve this issue. Since the first attempts, it appears that subcutaneous immunotherapy was associated with a higher risk of severe systemic reactions [5,6]. For this reason, other routes of administration were scanned and since allergen exposure via the oral mucosa seems to be tolerogenic, oral administration was explored [7]. For OIT, the approach generally follows the same principles of the immunotherapy of other allergic disorders and involves adminis-

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tering small increasing doses of food during an induction phase followed by a maintenance phase based on regular intake of a maximum tolerated amount of food [8]. In this regard, protein doses are increased over 1 day in rush protocols and continued over many weeks until a maintenance dose is reached. The maintenance dose is then taken daily for months to years [4].

Animal studies suggest that the high-dose consumption of an antigen results in the state of non-responsiveness due to anergy or deletion of antigen-specific T lymphocytes, whereas continuous low-dose ingestion may induce protective suppressive responses from Tregs [9,10]. On the contrary, intermittent consumption or nonoral exposures (e.g., cutaneous or inhalation) may induce IgE sensitization and allergic symptoms upon food ingestion [11]. The gut-associated lymphoid tissue is essential for normal tolerance to most foreign proteins, as well as in the immunologic response to oral immunotherapy [12,13]. The mechanisms of oral immunotherapy are mediated by intestinal dendritic cells, and cause a decrease in the concentrations of IgE-specific antibodies whereas IgG4 increase significantly. Secretion of the cytokines IL-10, IL-5, IFN- γ and TNF- α from peripheral blood mononuclear cells increases over a period of 6–12 months. In addition, T-cell microarrays show downregulation of genes involved in the apoptotic pathways [14].

An alternative route for immunotherapy for food allergies has been the sublingual route, which has already shown an effective strategy with aeroallergens in the treatment of severe allergic rhinitis and mild-to-moderate asthma [15,16]. SLIT typically involves food protein in the form of a liquid that is placed under the tongue for 2 min and then swallowed. Dose escalation and maintenance schedules are similar to those described for OIT, and the duration of treatment can be shorter due to lower target doses associated with SLIT [4]. SLIT usually requires a minor quantity of allergen, and has a better safety profile, possibly related to the high tolerogenic phenotype of oral APCs (Langerhans and myeloid dendritic cells). Oral tissues contain relatively few mast cells and eosinophils and, in comparison with subcutaneous or intestinal tissue, are less likely to give rise to anaphylactic reactions. Like for what happens with oral immunotherapy, sublingual-associated immune responses include the induction of circulating, allergen-specific Th1 and regulatory CD4⁺ T cells, leading to clinical tolerance [17].

The goal: tolerance or desensitization?

The goal of immunotherapy for food allergy is the achievement of tolerance or desensitization. Tolerance is the immunologic process that allows people to ingest the food without reaction even when the food is not

ingested for long periods (i.e., more than 1 month). Desensitization assures also the absence of allergic reactions, but the allergenic food must be ingested every day (or weekly) in order to maintain the absence of allergic reactions. Desensitization has been defined as ‘partial’ when the absence of allergic reactions is linked to smaller doses of proteins, and it is an appreciable result because it can protect against the ingestion of small or hidden quantity of allergen: milk, egg, peanuts are in fact used in the preparation of many foods [18].

The development of natural tolerance occurs normally in the first 2 years of life. It is an active immunologic mechanism in which the gut-associated lymphoid tissue becomes tolerant to the different epitopes present in the food [19,20] and is due to the production of antigen-specific Treg cells. In allergic patients, this process is not regulated and the production of IL-4, IL-5, IL-13 and specific IgE, through the Th2 cytokine network, is observed [21,22].

It must be underlined that, even in food allergic patients, it is not uncommon to develop a subsequent natural tolerance for the most frequent allergens with many children outgrowing their allergy [23]. In a study by Gupta and colleagues, the total frequency of tolerance acquisition was 26.6% at a mean age of 5 years, with higher frequency of tolerance acquisition for milk, egg and soy allergy (41, 40 and 36%, respectively). The probability of outgrowing the allergy is associated with a history of mild reactions, allergy to only one food, atopic dermatitis and lower specific IgE levels [24]. Patients who had experienced their allergic reactions later in life, were those with lower probability to outgrowing their allergy.

The development of the oral tolerance is the main goal of food immunotherapy, involving mechanisms substantially identical to what are seen in the development of natural tolerance. Tolerance involves Tregs, clonal deletion of T-cell subsets and allergen-specific anergy. As reported in several studies, these changes lead to the increase of hypoproliferative and nonreactive subsets of CD4⁺ T cells, and to the suppression of cellular or humoral responses. These changes can be observed after the ingestion of repeated low doses or single high doses of allergens. In particular, the frequent ingestion of small doses leads to the induction of antigen-specific Tregs with suppressor activity, including TGF- β -producing CD4⁺ T helper-3 cells (Th3) and gut-derived antigen-specific CD4⁺ CD25⁺ Foxp3⁺ T cells known as induced Treg (iTreg) [25]. In synthesis, development of tolerance involves an immunologic shift where Th2 responses diminish, Th1 as well as Treg responses strengthen. Specific serum IgE levels decrease and specific IgG4 levels increase in serum.

Different from tolerance, desensitization involves only transitory changes in the expression of effector cells (mast cells, basophiles), without modulation of the pathogenic immune mechanisms. The increase of food-specific IgG4 and the decrement of IgE antibodies titer, observed in many patients, is mainly temporary. For these patients a daily, uninterrupted ingestion of the food allergen is needed, also after the end of immunotherapy. When the allergen assumption is interrupted, the protective effect may be lost or significantly decreased. In addition, any factors affecting intestinal barrier function (i.e., exercise, gastroenteritis, stress) may cause loss of protection to the previously tolerated dose, even after the end of immunotherapy [26,27]. This is particularly important in pediatric population performing immunotherapy: incidence of food allergy is particularly high at this age, together with a higher risk of gastroenteritis and the frequent sporting activities. For this reason, this particular population has to be constantly followed up during the course of immunotherapy, and desensitized or partially desensitized patients have to be warned on the potential dangers of the loss of desensitization.

Immunological & clinical markers for immunotherapy

During the initial phases of food allergen-immunotherapy, allergen-specific IgE levels and IgE-mediated skin sensitivity wheal size often increase, although these levels decrease after 1–2 years of immunotherapy. In most studies of OIT, regardless of the variation between protocols, a significant rise (10–100-fold) in food-specific IgG4 levels has been observed. The role of this particular immunoglobulin in immune modulation is still controversial: it is thought that such noninflammatory and noncomplement binding isotype is able to capture the allergen before it reaches the effector IgE, preventing the proinflammatory cascade (activation of mast cells and basophils). The ‘pure count’ of IgG4 can be less important than their capacity to bind the inhibitor Fc- γ RIIB on APCs. The effectiveness could also be related to the decrease of IgE/IgG4 ratio, secondary to IL-10 upregulation, which can decrease IL-4-induced IgE switching while increasing IL-4-induced IgG4 production [26].

Studies of mast cell/skin tests during food immunotherapy generally correlate with analyses of peripheral blood basophils. Their activation is usually decreased after 3–6 months of immunotherapy, together with mast cell and skin test reactivity. Anyways, skin testing may have greater sensitivity compared with basophil histamine release, as demonstrated in several studies, in which basophil activation did not fall, but skin reactivity was correlated with desensitization to food [26].

For all the main protocols examined in this review, the results on IgE, IgG4, basophil activation and skin prick test are reported.

In a recent study, the effects of immunotherapy for food allergy on dendritic cell innate and adaptive immune functions in dendritic cell (DC) have been investigated. The results showed that SLIT decreased Toll-like receptor-induced IL-6 secretion by myeloid DCs (mDCs). SLIT and OIT altered mDC IL-10 secretion, a potent inhibitor of Fc ϵ RI-dependent proinflammatory responses. OIT uniquely augmented IFN- α and decreased IL-6 secretion by plasmacytoid DCs, which was associated with reduced Toll-like receptor-induced IL-13 release in pDC-T cell co-cultures. Both SLIT and OIT decreased Th2 cytokine secretion to CM in pDC-T, but not mDC-T, co-cultures. The results of this study suggested that SLIT and OIT have unique ways to reduce inflammatory response and the differences observed in the protocols can be explained by their different molecular pathways [28].

Clinical studies: the allergens

Milk

Cow milk (CM) allergy is the most frequent food allergy in childhood, with a prevalence of 2–3% of the infants [29–32]. Cow’s milk allergy is the food allergy presenting the highest natural tolerance acquisition rate: more than 80% of children presenting with milk allergy bring out their allergy by the age of 5 years, and non-IgE-mediated subtype even earlier than IgE-mediated [29]. However, children with higher CM-IgE titer, especially for casein, and low levels of CM-specific IgG4 are associated with persistent allergy. The most significant studies of OIT and SLIT for milk allergy are resumed in **Table 1**. Specific OIT for milk allergy has been tested since the first studies by the group of Patriarca [33,34], which showed encouraging results, and up to 80% of desensitized patients [34].

Since that time, a constantly growing number of studies has been carried out, and the results seem promising in terms of desensitization induction, while less clear data exist on the development of tolerance. In particular in 2004, in a small pilot study, Meglio and colleagues successfully desensitized 15 out of 21 children with severe IgE-mediated CM’s allergy, who, at the end of the study were able to tolerate 200 ml of milk, while three reached a partial desensitization, and three were not able to tolerate any dose of milk. In all the patients, sensitivity to skin prick test for casein and β -lactoglobulin decrease, but the level of IgE did not change [35].

The first study to assess the tolerance after a 2-month period of elimination of milk, was conducted by Staden and colleagues [37]. At rechallenge, 9/25 patients (36%)

Study	Year	Number of patients	Route	Tolerance assessment	Duration	Amount of milk during maintenance	Results	Side effects (symptoms)	Ref.
Patriarca <i>et al.</i>	1998	6 milk OIT	Oral	No	104 days	100 ml two- to three-times a week	100% DES		[33]
		5 elimination diet							
Patriarca <i>et al.</i>	2003	29 milk OIT	Oral	No	136 days	120 ml twice a week for 6–9 months	65.5% DES	51.5% of patients	[34]
		16 elimination diet					17.2% withdrawn		
Meglio <i>et al.</i>	2004	21 milk OIT	Oral	No	6 months	200 ml	71.4 % DES	61.9% of patients (oral, gastrointestinal and respiratory, one angioedema)	[35]
							14.3% PAR DES 14.4% No DES		
de Boissieu <i>et al.</i>	2006	8 milk SLIT	Sublingual	No	6 months	1 ml	75% DES	75% of patients (oral and respiratory)	[36]
Staden <i>et al.</i>	2007	14 milk OIT	Oral	Yes	21 months	250 ml	36% TOL	100% of patients in the rush phase (mostly cutaneous or gastrointestinal)	[37]
		11 egg OIT 20 controls					12% DES 16% PAR DES 36% No DES		
Longo <i>et al.</i>	2008	30 Milk OIT	Oral	No	10 days rush induction; then 150 ml for 1 year	150 ml	36% DES	>90% of patients (oral, cutaneous, gastrointestinal and respiratory)	[38]
		30 placebo					54% PAR DES 10% No DES		
DES: Desensitization; OIT: Oral immunotherapy; PAR DES: Partial desensitization; SLIT: Sublingual immunotherapy; TOL: Tolerance.									

Table 1. Milk immunotherapy protocols (cont.).

Study	Year	Number of patients	Route	Tolerance assessment	Duration	Amount of milk during maintenance	Results	Side effects (symptoms)	Ref.
Skripak <i>et al.</i>	2008	13 milk powder OIT	Oral	No	23 weeks	500 mg of powder (15 ml of milk)	92% in the active group significantly increased the milk threshold 46% DES	45.4% of doses (mostly oral and gastrointestinal)	[39]
		7 placebo							
Pajno <i>et al.</i>	2010	18 milk OIT	Oral	No	18 weeks	200 ml	67% DES 22% dropped out 22% No DES	80% of patients	[40]
Martorell <i>et al.</i>	2011	30 milk OIT	Oral	No	16 weeks	200 ml	90% DES 3.3% PAR DES 3.3% No DES 3.3% dropped out	47% of patients moderate reaction, 33% mild reaction (most commonly urticaria-angioedema and cough)	[41]
		30 placebo							
Keet <i>et al.</i>	2012	30 patients	Sublingual	Yes	12 weeks	7 mg	SLIT: 40-fold increase of the threshold; 10% TOL SLIT-then-OIT: 54–159 fold increase of the threshold; 40% TOL	29% of SLIT doses 23% of OIT doses OIT was more frequently associated to systemic reactions (gastrointestinal, respiratory)	[42]
		10 SLIT	Oral						
		20 SLIT-then-OIT							

DES: Desensitization; OIT: Oral immunotherapy; PAR DES: Partial desensitization; SLIT: Sublingual immunotherapy; TOL: Tolerance.

were tolerant, 3/25 (12%) were desensitized, 4/25 (16%) partially desensitized and 9/25 (36%) withdrew the study because of systemic reactions. The percentage of tolerant patients in the control groups who outgrew their allergy (natural tolerance) was 35%. A rush induction in hospital was attempted by Longo and colleagues [38], followed by a home build-up to 150 ml of milk. After 1 year, 36% of subjects were able to tolerate the full doses, 54% were partially desensitized (maximum of 50–150 ml of milk) and 10% were not able to tolerate it. A reduction of milk-specific IgE was found in half of the patients, mainly who initially had shown a level lower than 100 kUA/ml.

A different food vehicle (powdered milk proteins) was used by Skripak and colleagues in 20 children aged 6–17 years [39]. Twelve patients in the active group completed the treatment. After OIT, the median cumulative dose inducing a reaction in the active treatment group was 5140 mg, while all patients of the placebo group reacted at 40 mg. Side effects were more common in the active group (45.4 vs 11.2%), with oral symptoms being most common. Milk IgG4 levels increased significantly in the active treatment group, while specific IgE level did not change significantly.

Other protocols have been tested in the last years by Pajno and colleagues [40] and Martorell and colleagues [41], which respectively desensitized 67 and 90% of patients. In particular, the second study was performed in children aged 24–36 months and adverse reactions were frequent, especially in the skin, respiratory and GI tract and two subjects required epinephrine.

More recently, a Cochrane review took in consideration a total of 157 records of immunotherapy for milk allergy, but only 16 records, reflecting five randomized controlled clinical trials, were included in the revision [34]: the rates of desensitization ranged from 36 to 90%. The results were modest when patients with history of anaphylaxis or with higher IgE titer were included.

Sublingual route for milk allergy has been tested in fewer studies. The first attempt was performed by de Boissieu and colleagues [36] in an open-label pilot study performed in eight children with a target dose was 1 ml per day. Six patients completed the protocol, and an increase in the mean eliciting dose was observed in all of them, while no changes occurred in milk-specific IgE levels.

More recently, a larger study was conducted on 30 subjects [42]. The subjects were divided in two groups: the first performed only a SLIT protocol, while the other was a SLIT then crossing over OIT. In the SLIT group, an increase in the threshold of 40-fold after 60 weeks was observed and at the end of the study only one out of ten (10%) patient could tolerate an 8 g

oral food challenge. In the SLIT-then-OIT group, the increase of the threshold was higher (54 to 159-fold) and 8 out 20 (40%) achieved the tolerance. IgE levels decreased in all the patients, while the immune changes in specific IgG4 levels and skin prick test reactivity were more frequently observed in the SLIT-then-OIT group, with basophile activation decreasing only in this group. Adverse reactions were more common in the SLIT group (29 against 23%), but in the other patients were more severe.

In western countries, milk is widely consumed and to be desensitized (or tolerant) to more than 150 ml of milk could prevent from reaction even after the ingestion of high amount of milk (i.e., during the breakfast); the desensitization to a dose ranging from 25 to 150 ml is a considerable result, because it can prevent reaction of milk-containing food (i.e. biscuits, cheese). Lower levels of desensitization are too limited and cannot be preserved over the time or be easily lost in case of gastrointestinal infections or after intense exercise.

Egg

The first attempt at immunotherapy for egg allergy was performed by Patriarca and colleagues: in this first study immunotherapy was used to test different foods (i.e., milk, egg, fish, apple) and the rates of desensitization induction were encouraging [31]. Different studies and protocols performed since that time have been resumed in Table 2. In the first 10 years, small open label trials and uncontrolled studies included patients allergic to egg and other food (milk, peanuts), tolerance rate was not assessed, and the terms ‘desensitization’ and ‘tolerance’ were used indistinctly. The first author who specifically assessed the two states was Buchanan [43]: after a 24-month protocol 57% of patients were desensitized while only 28.6% were tolerant. Even Vickery and colleagues [44] assessed the tolerance rate: their results are the highest in terms of tolerance induction among all the studies of food immunotherapy (75%).

The ‘second generation’ of egg immunotherapy protocols was randomized, controlled clinical trials. Results were comparable among the different centers with rates of desensitization ranging from 69 to 92% [45,47], and lower rates of tolerance induction (28–36%) [37,48], a result very close to the acquisition of natural tolerance.

The outcomes of the studies were independent from food vehicle used (raw egg, pasteurized whole egg, pasteurized egg white) and the maintenance dosage was also unrelated with the results in terms of desensitization or tolerance induction.

On the contrary, the severity of egg allergy was a significant factor for the outcomes of the immunotherapy.

Table 2. Egg immunotherapy protocols.									
Study	Year	Number of patients	Route	Tolerance assessment	Duration	Amount of egg in maintenance	Results	Side effects (symptoms)	Ref.
Patriarca <i>et al.</i>	1998	5 OIT 4 placebo	Oral	No	4–5 months	One egg two-times a week	100% DES		[33]
Buchanan <i>et al.</i>	2007	7 OIT	Oral	Yes	24 months	0.3 g of egg proteins	57.1% DES 28.6% TOL	100% of patients (oral, cutaneous, gastrointestinal, respiratory)	[43]
Morisset <i>et al.</i>	2007	49 OIT 35 elimination diet	Oral	No	6 months	7 g of egg proteins	69.4% DES		[45]
Staden <i>et al.</i>	2007	11 OIT 10 elimination diet	Oral	Yes	67 days	2.8 g of egg proteins	36.7% TOL	100% of patients (mostly cutaneous or gastrointestinal)	[37]
Vickery <i>et al.</i>	2010	8 OIT	Oral	Yes	18–50 months	0.3–3.6 g of egg proteins	75% TOL	83% of patients during initial escalation day 50% during build-up; 0% during maintenance (mostly gastrointestinal and respiratory)	[44]
Itoh <i>et al.</i>	2010	6 OIT	Oral	No	15 days	7.5 g of egg proteins	100% DES	100% of patients (mild symptoms, mostly gastrointestinal, cutaneous, respiratory)	[46]
Fuentes-Aparicio <i>et al.</i>	2012	40 OIT 32 elimination diet	Oral	No	13 weeks	8 g of egg proteins	92.4% DES	52.5% of patients; 32.5% required treatment (gastrointestinal, respiratory)	[47]
Burks <i>et al.</i>	2012	40 OIT	Oral	Yes	22 months	1.6 g of egg proteins	10 months OIT = 55% DES	25% of doses	[48]
*Tolerance was assessed after 22 months of SOTI and 2 months of total egg avoidance. DES: Desensitization; OIT: Oral immunotherapy.									

Table 2. Egg immunotherapy protocols (cont.).

Study	Year	Number of patients	Route	Tolerance assessment	Duration	Amount of egg in maintenance	Results	Side effects (symptoms)	Ref.
		15 placebo					22 months OIT = 75% DES 24 months = 28% TOL [†]	After 10 months 8.3% (Mostly oral, pharyngeal, respiratory, gastrointestinal)	
Leonard <i>et al.</i>	2012	79 OIT 47 elimination diet	Oral	No	6–12 months	Two baked eggs per week (3.42 g proteins per day)	53% DES raw and baked egg 36% DES only baked egg	1.3% (gastrointestinal)	[49]
[†] Tolerance was assessed after 22 months of SOTI and 2 months of total egg avoidance. DES: Desensitization; OIT: Oral immunotherapy.									

In a protocol in which children with severe egg allergy (i.e., history of anaphylaxis or severe reactions after egg ingestion) were enrolled, no patients reached the maintenance dose but nine out of ten patients achieved a ‘partial desensitization,’ defined as the ability to eat a dose between 10 and 40 ml of egg [46].

Rush protocols, with build-up phases of 1 to 3 days, have showed similar rates of desensitized patients with respect to the conventional methods, but they are till now completely investigational and results are limited to few studies [46,50,51]. Duration of rush protocols is the most important advantage, but adverse reactions are common, in most of the cases limited to GI tract. For this reason, the initial build-up phases must be performed in a hospital setting.

In other studies, the role of heated egg in the immune tolerance has been investigated with positive results. More than 90% of egg-allergic patients are allergic to ovalbumin or ovomucoid and the first is degraded by heat, while the second remains intact. Patients allergic to only ovalbumin can tolerate heated or baked egg. It would be reasonable to think that only patients allergic to the ovomucoid do not experience symptoms after eating heated egg, but recent studies demonstrated that a lower IgE/IgG4 ratio (independently for ovomucoid or ovalbumin) is less commonly associated with reactions to baked egg [18], and baked or heated egg is tolerated by most of the patients affected by egg allergy, as reported by Turner and colleagues [52]: in their experience 64% of egg-allergic patients passed a challenge with baked egg, while 36% reacted and 14% presented with anaphylaxis. Two different studies demonstrated that the ingestion of low doses of baked egg causes an acceleration in the development of tolerance [49,53], while another [54] reported that frequent consumption of baked egg is not associated with a different rate of decline in skin prick test size to egg.

Egg is an important source of proteins (10–15 g) and largely consumed in the world, alone or for preparation of different products and its traces can be found easily in the food. The achievement of a partial desensitization to egg protein (1–5 g) can be useful to prevent the risk of anaphylaxis after ingestion of hidden amounts of egg in food (i.e., one biscuit), while it cannot protect against high amount of this food (i.e., one scrambled egg), but it must be considered that it is uncommon that an egg-allergic subject eats an entire egg, while it is easy that an egg-allergic child eats a hidden egg in particular food (i.e., a cake). Subjects tolerant to more than 5 g of proteins can presumably consume the recommended amount of egg in the diet (two eggs per week).

Peanut

In the last 20 years, peanut OIT has been reported in anecdotal studies, which had significant methodologi-

cal concerns, including small sample sizes, selection bias and lack of control groups or pre-OIT challenge as reported in **Table 3** [55].

The largest study published so far was the STOP II trial by Anagnostou and colleagues [63], which was performed in 99 children aged 7–16 years in an open-label placebo-controlled clinical trial. Desensitization rate was 91% in the active group, while none in the control group was desensitized. Adverse events, reported also in the control group, were mild and in only two cases the symptoms needed epinephrine (both in the same patient).

In the placebo-controlled study by Varshney and colleagues, 28 patients aged 1–18 years were enrolled. In the active group, after 48 weeks, 16 out of 19 (84%) were successfully desensitized, while the placebo group achieved a poorer result, with a lower median cumulative dose (280 mg vs 5 g). The OIT patients reported a decreased skin prick test wheal, an increased level of allergen-specific IgG4 and a decreased production of IL-5 and IL-13, while no changes were seen in the IgE levels [59]. In the previous years, other studies had tested peanut immunotherapy, without control groups [56–58,60]. In all of them, the majority of patients achieved the ability to tolerate the maintenance doses, and 94% of these passed the final double-blind, placebo-controlled food challenge [64]. Tolerance was assessed only in one study, even if after a short period of allergen-free diet (15 days): 11 out of 14 patients (79%) tolerated the 500 mg maintenance doses, while 3 tolerated lower doses [58]. Confirming the findings of Varshney and colleagues, IgE levels remained constant during the protocols, while IgG4 increased and Th2 cytokine production decreased [56–58,60].

The sublingual route for peanut allergy immunotherapy has been tested in two studies. In the first [61], a randomized study involving 18 patients, the oral food challenge performed at 1 year revealed that in the active group, the maximum cumulative dose was 1710 mg of peanut protein, while in the placebo group it was 85 mg. At the end of the study, SLIT patients presented a smaller SPT wheal diameter, decreased specific IgE and IL-5 production, increased specific IgG4.

A more recent multicenter, double blind, placebo-controlled trial was performed in 40 patients (median age 15 years) [62]. In the first 44 weeks, the study was blinded, and at the end of this time, the patients underwent to a 5 g oral food challenge: 14/20 of the patients in the active group could tolerate a tenfold higher dose comparing to the beginning of the trial, while no one could tolerate the maximum doses. The responders continued to assume SLIT for other 24 weeks (total 68 weeks), and the prolonged SLIT increased the successfully consumed dose. Regarding the placebo

group, after the 44th week, 17 patients underwent to a high-dose SLIT for another 44 weeks and 44% of these patients were considered responders. The patients did not show an appreciable decrement in specific IgE value, with a modest increase of specific IgG4. The responders presented a smaller skin prick test wheal diameter in comparison with the nonresponders. Side effects were uncommon.

Comparing sublingual route with oral after 2 years of treatment, OIT produces greater immunologic changes than SLIT in peanut allergy. Changes in IgE/IgG4 ratio, and peanut-specific IgG and IgE are less evident after SLIT and the eliciting dose thresholds in the double-blind, placebo-controlled food challenge are lower [65].

Peanuts are consumed worldwide and can be eaten raw, used in recipes, made into solvents and oils, medicines, textile materials and peanut butter. The percentage of proteins in this food is lower than egg (25–30%). One peanut of 1 g contains roughly 250 mg of proteins and for this reason, the only study on peanut allergy to test a high quantity of proteins has been the one by Varshney (56), in which the amount during maintenance was 4 g (approximately 16 peanuts). In the other studies, the amount was limited, and the desensitized (or tolerant) patients result protected only against accidental ingestion of less than four to six peanuts (especially useful in childhood).

Other food

Hazelnut allergy has been treated in a double-blind placebo-controlled SLIT in Spain in 23 adults, 12 in the active group [66]. Build-up phases had a duration of 4 days, while maintenance (188 mcg of Cor a 1 and 122 mcg of Cor a 8 proteins) lasted 5 months. The patients of the active group presented an increased threshold dose in comparison with the control group, there was no change in the hazelnut-specific IgE levels compared with baseline, while specific IgG4 and IL-10 increased significantly. Systemic reactions occurred only in 0.2% of the doses subministrated.

Another randomized study with SLIT was performed for peach allergy, with 37 adults receiving treatment and 19 placebo. The build-up phases were performed in 3 days in a hospital setting, while the maintenance had a duration of 6 months with a three-times per week dosing. Pru p 3 (peach lipid transfer protein) was the protein given to the patients, with a maximum of 10 mcg per dose. In the active group, a nine-times raise of the threshold for eliciting reaction than the baseline was observed. Side effects were mostly local and system reactions were present only in 1.2% of the total dosing. In this study, specific IgE level decreased [67].

Table 3. Peanut immunotherapy protocols.

Study	Year	Number of patients	Route	Tolerance assessment	Duration	Amount maintenance	Results	Side effects (symptoms)	Ref.
Jones <i>et al.</i>	2009	29 OIT	Oral	No	36 months	1800 mg peanut proteins	93.1% DES (3.9 g of peanut protein during challenge)	92% of patients in the initial day (most commonly upper respiratory symptoms) 46% of doses during maintenance	[56]
Clark <i>et al.</i>	2009	4 OIT	Oral	No	8 weeks	800 mg peanut proteins	OIT conferred protection against the ingestion of at least ten peanuts in all the patients	75% of patients, especially in the first phases of OIT (oral, cutaneous, respiratory)	[57]
Blumchen <i>et al.</i>	2010	23 OIT	Oral	Yes [†]	9 weeks	500 mg peanut proteins	78.6% TOL 21.4% PAR DES	7.9% of rush phase doses (mostly gastrointestinal and cutaneous) 2.6% of maintenance doses (mostly respiratory)	[58]
Varshney <i>et al.</i>	2011	19 OIT 7 placebo	Oral	No	48 weeks	4 g peanut proteins	84% DES	47% of patients in the rush phase; 1.2% of doses during build-up and maintenance	[59]
Anagnostou <i>et al.</i>	2011	22 OIT	Oral	No	32 weeks	800 mg peanut proteins	64% DES	86% of patients (mild symptoms)	[60]
Kim <i>et al.</i>	2011	11 SLIT	Sublingual	No	12 months	200 µg peanut proteins	The treatment group safely ingested 20-times more peanut protein than the placebo group	11.5% of doses (mostly oral itching)	[61]
Fleischer <i>et al.</i>	2013	20 SLIT 20 placebo	Sublingual	No	68 weeks	165–1386 µg peanut proteins	70% of the active group could tolerate a 10-fold higher dose of peanut	36.9% of doses (mostly oropharyngeal symptoms)	[62]
Anagnostou <i>et al.</i>	2014	39 OIT 46 placebo	Oral	No	26 weeks	800 mg peanut proteins	61.5 % DES in active group	6.3% of doses mouth itch; 5.6% of doses gastrointestinal symptoms; 0.8% of doses respiratory tract symptoms	[63]

[†]Tolerance was assessed after a short period of allergen-free diet (15 days).
DES: Desensitization; OIT: Oral immunotherapy; SLIT: Sublingual immunotherapy; TOL: Tolerance.

OIT for wheat allergy has been practiced in a recent pilot study (six patients), with promising results: at the end of the study (6 months) five patients (83%) could tolerate a 100 g of wheat daily, together with rye and oat. Specific IgE for wheat initially increased, and at the end of the study decreased. Adverse reactions were mild and reported in only 6.25% of doses [68].

Since today, there have been no reports of immunotherapy for fish or shellfish allergy, a serious kind of disease in which spontaneous tolerance is rarely reached and allergy persists throughout the life. Neither soy nor wheat allergy have found an immunotherapeutic treatment so far.

Future horizons

Skin is an important immune organ eliciting innate and adaptive immune functions [69]. Recently, some studies have suggested that a patch containing food allergen (peanut) could activate Langerhans cells, with migration to lymph nodes and downregulation of effector cell responses. Preclinical studies have shown that allergens can be taken up and internalized by dendritic cells of the skin and transported to the draining lymph nodes more rapidly in sensitized than in naive mice. Repeated application downmodulated the immune responses and generated clearly tolerogenic Treg cells. Interestingly, this tolerogenic effect disappeared when EPIT was carried out on stripped skin [14,70].

Epicutaneous immunotherapy has been tested for milk and peanut allergy. A double-blind placebo-controlled study was performed by Dupont and colleagues [71], who administered a 48-h patch with milk powder (three per week) for a total of 3 months. At the end of the protocol, the cumulative reactive dose of milk was higher in the active group than in the placebo. Apart from local reaction (eczema, erythema), no important adverse reaction was reported.

In peanut allergy, a large study has been conducted in the US, enrolling 100 patients (80 active groups, 20 controls), using a patch containing peanut proteins (250 µg in children, 500 µg in adolescents and adults) for 2 weeks, with the aim to test the safety of this procedure. Only 2/80 children experienced severe adverse reactions and abandoned the protocol, while 90% showed moderate local reactions [72].

A peanut efficacy trial (ARACHILD), double-blinded and placebo-controlled, enrolling 54 children with severe peanut allergy (aged 5–17 years) has shown that children present a consistent and sustained desensitization, with up to 67% responders [73]. Nowadays, other studies have been initiated and are still ongoing.

Modified allergens or tolerogenic peptides, ultimately optimized for human leukocyte antigen background of the patient, are at present explored for tolerance induc-

tion, whereas anti-IgE antibody (omalizumab) may be used to improve OIT safety and efficacy [74,75]. Molecular preclinical studies have shown that TH1 polarizing agents combined with modified allergen packaged in nanoparticles, IGF-2, intravenous immunoglobulin, Tregitopes or allergen encased oligomannose-coated liposomes may be important for clinical tolerance induction.

Moreover, probiotics use seems to be promising, being these agents able to modulate the mucosal immune response, and clinical trials demonstrated that specific strains, especially lactic acid bacteria ones, reduce allergic symptoms and may favor good outcomes of immunotherapy [76].

Safety of immunotherapy for food allergy

In all the protocols of immunotherapy, side effects are quite common. Their occurrence ranges from 5 to 100% of patients, independently from the food chosen (Tables 1–3). The initial phases of immunotherapy are more likely to produce side effects, especially in the first days of rapid increment of allergen, and rush protocols are usually more dangerous. A higher occurrence of systemic reaction has been observed in OIT, while oral reactions are quite common in SLIT. The reported incidence of side effects varies from the districts involved. Cutaneous symptoms (such as itching, erythema, urticaria) are common, together with local symptoms (oral and pharyngeal itching). Gastrointestinal and respiratory symptoms may be severe, and are usually linked with withdrawals from the study and poor adherence to the protocols: usually they comprise nausea, vomiting, abdominal pain or asthma and rhinitis. The occurrence of anaphylaxis is purely anecdotal and reported in very few cases.

In a small pilot study, premedication with anti-IgE antibody omalizumab before and during OIT for milk allergy has reduced the incidence of side effects to 1.8%, mostly mild [75].

Conclusion

At present, immunotherapy is not the gold standard for the treatment of food allergy. In fact immunotherapy for food allergy is still not ready for the clinic, but current and upcoming studies are dedicated to collect enough evidence for the possible implementation and standardization of immunotherapy for food allergy, together with a standard treatment for the different foods involved.

Finally, investigation that conclusively demonstrates OIT is efficacious and safe is still missing, and so for the definition of intermediate and long-term outcomes. However all the studies presented in this review have demonstrated that both OIT and SLIT, and more

recently EIT have been able to desensitize patients. OIT patients have achieved generally a higher desensitization (and tolerance) rate, probably due to the higher doses of allergens that can be ingested.

It must be underlined that for food allergies, the protocols have been tested in small groups of patients, particularly selected in terms of severity of the disease and IgE values.

As recently reported, a promising strategy for preventing adverse reactions and IgE cross-linking, enhancing safety of immunotherapy, while still activating T cells, is the use of tolerogenic peptides. The implementation of this immunotherapy approach has the potential of not only increasing the chance of achieving a permanent state of tolerance, but also improving the safety and tolerability of the therapy [77].

Future perspective

In the next 5–10 years, an increasing number of protocols for food immunotherapy are expected. Other foods (such as wheat, peach, hazelnut) are expected to be tested in larger double-blinded, placebo-controlled

clinical trials, together with new and safer routes (lymphatic, epicutaneous). The incidence of adverse events will be probably reduced by the contemporary use of premedication (antisthamines, anti-IgE antibodies).

Evaluation of tolerance through specific immunomarkers (CD4⁺ hypoproliferative subsets of cells, CD63⁺ cells) will be one of the goals of the research in the future. It is also expected a 'pre-protocol identification' of the immune system of the patients, which will allow the allergologist to select the right patients in whom immunotherapy could be more efficacious.

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Executive summary

- Food allergy affects 2–10% of people and the only treatment is total food avoidance, which is often difficult to obtain; immunotherapy could represent the only definitive treatment for this kind of allergy.
- The two most employed routes of immunotherapy for food allergy are oral immunotherapy (OIT) and sublingual immunotherapy (SLIT): first the amount of allergen is higher, together with a higher incidence of systemic side effects. Sublingual immunotherapy is moderately safer, but the amount of allergen is limited.
- OIT and SLIT have both demonstrated appreciable results in terms of desensitization induction, with roughly 70–90% of patients being desensitized at the end of the protocols; tolerance acquisition after immunotherapy is less common, and the results are similar to those of natural tolerance (around 30–50%).
- Immunological markers are the reduction of IgE (after an initial increase), the increment of IgG4 and the overall reduction of IgE/IgG4 ratio. During and after immunotherapy, basophil and mast-cell activation is diminished, but the size of skin prick test is overall more useful and precise in clinical practice to determinate the effects of immunotherapy.
- Milk, egg and peanuts OIT and SLIT have been tested more than other foods since these are the three most common food allergens. The results are comparable and show a high rate of desensitization induction and a lower tolerance acquisition, this last being assessed rarely. In the present days, other food trials are limited to pilot studies.
- Future directions are on the employment of the epicutaneous immunotherapy, modified allergens or tolerogenic peptides, anti-IgE antibody omalizumab, TH1 polarizing agents combined with modified allergen packaged in nanoparticles, IGF-2, intravenous immunoglobulin, Tregitopes or allergen-encased oligomannose-coated liposomes and probiotics.
- Side effects are quite common in the initial phases of immunotherapy and usually limited to cutaneous or local (oral) reactions. Respiratory and gastrointestinal reactions can be various, from vomiting and abdominal pain to rhinitis and asthma. Severe reactions (i.e., anaphylaxis) are rare and often limited to patients with severe allergy (i.e., low threshold at the double-blind, placebo-controlled food challenge, high IgE levels).

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