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REVIEWS**

# Immunotherapy: a new horizon for egg allergy?

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Egg allergy is the second most frequent food allergy in children of the Western Countries, with an overall prevalence of 1–3%. Today strict avoidance diet is the only treatment, but its feasibility is difficult to obtain in childhood because of the large amount of egg proteins present in different foods. From 1998, a growing number of protocols on immunotherapy for egg allergy have been published, but all of them differ for patients' age, inclusion of high-risk patients, amount of allergen administered, duration of the protocols and presence of a control group. We reviewed the protocols performed in the last 15 years, to underline the most important issues in this kind of food immunotherapy, and the rates of tolerance or desensitization induction.

**KEYWORDS:** allergy • desensitization • egg • oral immunotherapy • review

Egg allergy affects 1–3% of children in the Western countries, and it is believed to be the second most common food allergy, after cow's milk protein allergy [1,2]. Almost two-thirds of pediatric patients affected by egg allergy heal spontaneously within the age of 16 years, although recent observations have shown a significant persistence over the second decade [3]. In these patients, symptoms are usually more severe and IgE level is higher (>50 IU/l), especially those directed against linear epitopes of the ovomucoid (OM Gal d 1), the major allergen of egg (34–97% of subjects). This protein, composed of 186 amino acids and resistant to digestion and heat, is divided into three domains, all potentially capable of binding to IgE and trigger allergic reactions. In an experimental murine model, a modified isoform of the third domain of ovomucoid was used to treat mice artificially sensitized against intact ovomucoid. Among the results of the study, there were a complete suppression of anaphylactic symptoms and lower serum histamine levels, significantly reduced ovomucoid-specific IgE and IgG<sub>1</sub> levels and enhanced specific IgG and IgG<sub>2a</sub> levels [4]. Ovalbumin (OM Gal d 2) is the most abundant protein in hen's egg, representing up to 60% of egg's proteins. Its allergenicity is strongly reduced by thermal treatment [1]. Other allergens present in egg are the lysozyme and two minor egg white (EW) proteins, identified as ovomucoid inhibitor and clusterin, which were found to react with serum IgE from egg-allergic patients [5].

Today, the avoidance of the food is the only preventive treatment, but this strategy can cause a poor quality of life; moreover, a high incidence of adverse events have been described, because of the presence of unnoticed egg components in different foods [6]. Based on positive results of immunotherapy for cow's milk protein allergy [7], a growing number of studies have been performed also for egg allergy [3]. The limited number of patients treated, the differences between the studies and the absence of shared guidelines makes this issue intricate, with different aspects still to be clarified.

## Egg allergy immunotherapy

All immunotherapy protocols are performed in IgE-sensitized patients, with history of allergic reactions after ingestion of egg proteins and in most part of the cases with a positive double-blind placebo-controlled food challenge (DBPCFC). Protocols are usually characterized by a one-day rapid build-up phase, a slow build-up phase and a maintenance phase, of variable duration, in which a high amount of allergen is daily ingested. First doses are prepared and given in a hospital setting, and are generally in the range of few milligrams (0.1–10), with the doubling of the doses every 15–30 min. Duration of build-up usually ranges from 1 to 4 months, and is characterized by a slow increment of dosage, usually every week or bi-weekly. Build-up phase doses are given with

pre-weighted containers or prepared by the families instructed by the physicians, and so the maintenance doses. Maintenance can last from 6 to 12 months, but it can be 'naturally' prolonged for years if the food allergen is consumed regularly. Rush protocols are faster and the maintenance dosage can be reached after a very short build-up (1–14 days) [3].

At the end of the protocol, a food challenge is performed to evaluate the acquisition of desensitization. Desensitization can be demonstrated with oral food challenge, while the subject is still receiving treatment or eating regular doses of a food, and tolerance is confirmed by performing oral food challenge after a mandatory discontinuation of food ingestion for a period of at least 2–4 weeks, although the length of the period of discontinuation has not been exactly defined [8].

Frequently, during oral immunotherapy, premedication drugs, such as antihistamines, are given to patients, in order to reduce the frequency of allergic reactions to the allergen. The opportunity of premedication during immunotherapy treatment has been debated in the past years. Their usage could be omitted in slow protocols and in low-risk patients, whose specific IgE levels are below 7 KUa/l [9]. Premedication is generally performed in patients with a past history of severe reactions and during rush protocols, in which maintenance doses are reached in 5–15 days [10,11]. Usual drugs employed as premedication are antihistamines, while in some cases montelukast has been used [12].

In this review, we performed a comparison of the recent published oral immunotherapy (OIT) protocols, performing a research of the words 'egg' 'immunotherapy' 'protocols' 'desensitization' 'specific oral tolerance induction' in PubMed database, with particular interest in the amount of proteins that patients consumed and in different food vehicles (i.e., raw egg, pasteurized egg, powdered egg or white-egg), evaluating the duration of the protocols, presence of control groups, classes of severity of the patients treated, rates of desensitization or tolerance acquisition.

### **The pursuit of tolerance**

The aim of immunotherapy to egg allergy is to induce a permanent tolerance or alternative desensitization, in order to allow patients to eat this food (or small, hidden doses) without risk of allergic reaction and to regularly ingest an important source of proteins in childhood.

Natural oral tolerance is what happens normally in the first years of life, after the ingestion of different foods in the period of the weaning and it is assured by gut-associated lymphoid tissue. First described by Wells and Osborne more than 100 years ago [13], it has been shown to be an active immunologic process and not merely the absence of an immune response to orally administered antigens [14]. Commonly, the production of antigen-specific Treg cells generally follows the ingestion of food antigens [15]. In food-allergic patients, the dominant immune response is characterized by the production of Th2-skewed T cells, triggered by IL-4 and resulting in the production of effector cytokines IL-4, IL-5 and IL-13, specific IgE [16] and changes in *CD63* expression in basophils [17].

The development of natural tolerance in children affected by food allergy has been reported in a recent study by Gupta and colleagues [18], in which 1245 cases of outgrown food allergy were identified. The total frequency of tolerance acquisition was 26.6% at a mean age of 5.4 years. Children with egg allergy had a higher frequency of tolerance acquisition (40.2%), similar to milk (41.1%) or soy (35.7%) allergy. The outgrowing of allergies was associated with a history of mild-to-moderate reaction, allergy to only one food, atopic dermatitis as unique symptom of allergy and white race. Probability of natural tolerance acquisition was significantly higher in patients who had experienced their first allergic reaction at younger ages and was lower for patients with first reactions occurring later in life, irrespective of allergen, severity or presentation.

In immunotherapy, desensitization describes the ability to ingest a food without reaction with continuous ingestion to regular doses of food. This state is mediated by changes in effector cells (mast cells, basophils), without modulation of pathogenic immune mechanisms. In contrast, tolerance is a state of total unresponsiveness to a food allergen and does not only involve effector cells, but also Treg or other T-cell subsets and/or allergen-specific anergy and clonal deletion [14]. In a recent research [19], the acquisition of tolerance in children after oral immunotherapy was reported to be accompanied by a decrease in the effector memory T-cells population and the increase in a particular subset of CD4<sup>+</sup> T cells with a hypoproliferative and non-reactive phenotype: these cells could constitute a marker of the development of oral tolerance. Both the ingestion of a single high dose or repeated low doses of allergens has been demonstrated to induce tolerance. In the first case with the stimulation of deletion of antigen-specific T-helper cells, Treg seem to be resistant to deletion [20,21]. After repeated low doses of allergens, the induction of antigen-specific Treg with suppressor activity has been observed: among these, TGF- $\beta$ -producing CD4<sup>+</sup> Th3 cells and gut-derived antigen-specific CD4<sup>+</sup> CD25<sup>+</sup> Foxp3<sup>+</sup> T cells known as induced Treg [22].

Unlike desensitization, oral tolerance does not need a continuous ingestion of allergen to be preserved. For this reason, tolerance acquisition is generally assessed after a variable period of discontinuation of allergen ingestion. After OIT, patients who develop tolerance are generally fewer than the desensitized patients. In this last group, 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 OIT. Possible mechanisms of oral desensitization include increased food-specific IgG<sub>4</sub> and decreased food-specific IgE antibodies, and reduced activation of mast cells and basophils [23]. Desensitization to a lower amount of proteins represents also a significant result, because it can protect against accidental ingestion of egg and it can be preserved over the time with regular intake of such foods [24].

**Table 1. Non-randomized or uncontrolled trials for egg oral immunotherapy.**

| Study (year)                   | Protocol duration | Type of study       | Food vehicle | Age range  | Patients (n)        | Maintenance dosage           | Results                | Ref. |
|--------------------------------|-------------------|---------------------|--------------|------------|---------------------|------------------------------|------------------------|------|
| Patriarca <i>et al.</i> (1998) | 4–5 months        | Open clinical trial | RE           | 3–15 years | 5 OIT<br>4 controls | 1 egg/2–<br>3-times a week   | 100% DES               | [25] |
| Buchanan <i>et al.</i> (2007)  | 24 months         | Uncontrolled trial  | PEW          | 1–7 years  | 7 OIT               | 0.3 g of egg<br>proteins     | 57.1% DES<br>28.6% TOL | [26] |
| Vickery <i>et al.</i> (2010)   | 18–50 months      | Uncontrolled trial  | PEW          | 3–13 years | 8 OIT               | 0.3–3.6 g of<br>egg proteins | 75% TOL                | [28] |

DES: Desensitization; OIT: Oral immunotherapy; PEW: Powdered egg white; RE: Raw egg; TOL: Tolerance.

### Open-label trials & uncontrolled studies

Especially in the first 10 years (1998–2007) of OIT for egg allergy, studies included patients allergic to different foods (milk, eggs, peanuts), and the results were often reported for allergic cohorts indifferently. The acquisition of tolerance wasn't generally assessed, preferring the evaluation of the desensitization, and furthermore in many studies, the terms 'desensitization' and 'tolerance' were used indistinctly. OIT for egg allergy was performed in the same centers or by the same authors who had performed immunotherapy for other foods, in particular milk. These protocols are summarized in TABLE 1.

The first attempt of egg immunotherapy was performed in a small open-label trial by Patriarca and colleagues [25], who proposed a standardized sublingual/oral desensitization program for food allergy in children, including milk, egg, fish and apple, with a success rate in 100% of patients for sublingual raw egg (with a maximum dose of one whole egg in 4 months). Tolerance rate was not assessed and the authors concluded that oral desensitization treatment could represent a safe and convenient alternative to strict-avoidance in the management of food-allergic subjects [24].

Buchanan and colleagues [26] reached a maintenance dose of 300 mg of egg proteins and maintained it for 24 months, in children aged 1–7 years; 4/7 (57.1%) patients achieved desensitization (no reaction after the ingestion of 8 g of egg proteins at the end of OIT), while 3/7 (42.9%) experienced symptoms during the challenge. This was the first study to assess the rate of tolerance acquisition: after 3 months of egg-restricted diet, another challenge was performed to evaluate tolerance in the four desensitized patients: two of them demonstrated oral tolerance, while the other presented symptoms (i.e., urticaria, abdominal pain, vomiting, periorbital edema) during the challenge. At the end of the study, all the seven patients were anyway able to ingest higher amounts of egg compared with the baseline, raising the threshold of food ingestion causing allergic symptoms on accidental egg ingestion [27].

In a subsequent open-label clinical trial, Vickery and colleagues [28] tested immunotherapy with EW powder in eight egg-allergic children, aged 3–13 years. To assess clinical tolerance, patients underwent DBPCFC 1 month after the conclusion of the protocol, which was classically subdivided into induction, build-up and maintenance (300 mg) phases, with

further increasing of 600 mg every 4 months to a maximum dose of 3600 mg, in subjects whose IgE levels were <2 kU/l. Two subjects withdrew the study before reaching the maintenance dose, and the six patients (75%) who reached at least the 300 mg of maintenance developed clinical tolerance to egg, assessed after a period of 1 month of egg-free diet. The median wheal diameter on EW skin prick testing decreased from 10 to 2.5 mm during OIT. Both EW and ovomucoid-IgE levels significantly decreased during the study, and corresponding IgG<sub>4</sub> levels increased. Transient increases were seen in egg-induced IL-10 and TGF- $\beta$  levels, while IL-13:IFN- $\gamma$  ratio decreased. At the present day, it is the study in which the amount of tolerant patients was higher (75%), even if the sample was small.

### Randomized placebo-controlled clinical trials

After the positive results of the first attempts of egg OIT, a constantly growing number of studies have been performed in the last years, with the inclusion of control groups of untreated patients and the randomization of the groups, trying to raise the amount of proteins and reducing the duration of immunotherapy, using different food vehicles and including high-risk patients with history of anaphylaxis. These protocols are summarized in TABLE 2.

The validity of the results of OIT is still a matter of debate, because there are many differences among the protocols in terms of kind of egg used and quantity of proteins. Even the number of subjects recruited has been different among the studies, ranging from fewer than 20 patients to 50–100 per protocol.

### Unblinded randomized placebo-controlled clinical trials

A larger study by the group of Patriarca and colleagues [29] was performed in 2007, using a higher amount of raw shaken egg (50 ml/day at the end of maintenance phase). The study comprised a control group of 10 patients who underwent to a strict elimination-diet. At the end of the protocol, 10 of 14 (71%) egg-allergic patients successfully developed desensitization upon challenge with egg yolk or albumen, 2 patients developed partial desensitization, defined as the possibility to eat small amount of egg included in other foods such as ice-cream, cakes or biscuits, while other 2 abandoned the protocol. The study also included three patients allergic to the albumen and two

**Table 2. Randomized, controlled clinical trials.**

| Study (year)                          | Protocol duration | Type of study               | Food vehicle | Age of patients             | Patients (n)                  | Maintenance dosage                       | Results                                                                                | Ref. |
|---------------------------------------|-------------------|-----------------------------|--------------|-----------------------------|-------------------------------|------------------------------------------|----------------------------------------------------------------------------------------|------|
| Patriarca <i>et al.</i> (2007)        | 140 days          | Non-blinded clinical trial  | RE           | 3–16 years                  | 14 OIT<br>4 elimination diet  | 6.3 g of egg proteins (50 ml shaken egg) | 71% DES                                                                                | [29] |
| Morisset <i>et al.</i> (2007)         | 6 months          | Non-blinded clinical trial  | RE           | 1–8 years                   | 49 OIT<br>35 elimination diet | 7 g of egg proteins                      | 69.4 % DES in OIT group<br>51.4% DES in avoidance group                                | [31] |
| Staden <i>et al.</i> (2007)           | 67 days           | Non-blinded clinical trial  | PE           | 0.6–12.9 years <sup>†</sup> | 11 OIT<br>10 elimination diet | 2.8 g of egg proteins                    | 36% TOL                                                                                | [30] |
| Fuentes-Aparicio <i>et al.</i> (2012) | 13 weeks (4–28)   | Non-blinded clinical trial  | PE           | 5–15 years                  | 40 OIT<br>32 elimination diet | 8 g of egg proteins                      | 92.4% DES                                                                              | [19] |
| Leonard <i>et al.</i> (2012)          | 6–12 months       | Non-blinded clinical trial  | BE           | 1.6–15.8 years              | 79 OIT<br>47 elimination diet | 2 Baked egg/week (3.42 g proteins/day)   | 53% DES raw and baked egg<br>36% DES only baked egg                                    | [49] |
| Burks <i>et al.</i> (2012)            | 22 months         | Double-blind clinical trial | PEW          | 5–11 years                  | 40 OIT<br>15 placebo          | 1.6 g of egg proteins                    | 10 months OIT = 55% DES<br>22 months OIT = 75% DES<br>24 months = 28% TOL <sup>‡</sup> | [37] |
| Dello Iacono <i>et al.</i> (2013)     | 6 months          | Non-blinded clinical trial  | RE           | 5–11 years <sup>§</sup>     | 10 OIT<br>10 elimination diet | 5 g of egg proteins                      | No patients reached maintenance dosage                                                 | [33] |
| Meglio <i>et al.</i> (2013)           | 6 months          | Non-blinded clinical trial  | RE           | 4–14 years                  | 10 OIT<br>10 elimination diet | 3.1 g of egg proteins (25 ml egg)        | 80% DES                                                                                | [32] |

<sup>†</sup>This range of age include also patients with cow milk's allergy.

<sup>‡</sup>Tolerance was assessed after 22 months of SOTI and 2 months of total egg avoidance.

<sup>§</sup>Children with very severe egg allergy.

DES: Desensitization; OIT: Oral immunotherapy; PE: Powdered egg; PEW: Powdered egg white; RE: Raw egg; TOL: Tolerance.

out of three could ingest albumen after OIT, while one out of three presented partial desensitization. All children under elimination diet had a positive DBPCFC at the end of the study. After the study, the patients were asked to continue to eat egg twice a week, and also in this second work the results in terms of tolerance acquisition was not assessed.

A protocol with higher doses of allergens for milk- and egg-allergic patients was performed by Staden and colleagues [30]. OIT was performed at home with fresh CM or lyophilized hen's egg proteins (maximum 2800 mg of proteins). In order to evaluate persistence of induced oral tolerance, children in the OIT group received a secondary elimination diet for 2 months after immunotherapy before another challenge. Twenty-five allergic patients (11 to hen's egg, 14 to CM) completed the protocol; 9/25 patients (36%), indistinctly classified as milk- or egg-allergic patients, acquired 'permanent tolerance', defined as the ability to

pass DBPCFC at the end of the protocol and after a 2-month avoidance period. This group of tolerant children was almost equal to what was obtained in the control group (35%), and the authors questioned if it was the natural course or a consequence of the OIT. The number of patients who passed the test after the protocol and who weren't able to pass it after the elimination diet was 3/25 (12%), while the 'partial responders' group, which comprised patients tolerating a food dosage lower than the planned full maintenance dose, was 4/25 (16%). The other nine patients (36%) were no responders, presenting symptoms during OIT, which was therefore stopped in the early phases. Allergen-specific IgE decreased significantly both in children who developed natural tolerance during the elimination diet ( $p < 0.05$ ) and in those with OIT ( $p < 0.001$ ). The authors stated that the advantages of OIT were the increased threshold dose for allergic reactions and the substantially reduced risk of severe allergic

reactions after accidental ingestion of the allergen. In this study, the rates of tolerance acquisition were not assessed.

A larger study was performed in the same year by Morisset and colleagues [31], who enrolled 90 children with egg allergy (aged 12 months to 8 years), randomized for avoidance or oral desensitization. Six months later, a single-blind placebo-controlled food challenge was performed with 7 g of raw egg white (REW). The single-blind placebo-controlled food challenge was positive in 30.6% of OIT patients versus 48.6% in the control group ( $p < 0.01$ ). After 6 months, in the group of the treated patients, the mean size of the prick test and the level of specific IgE were significantly reduced compared with the avoidance group. In this last group, the threshold of reactivity was lower and more severe symptoms were observed.

In 2012, Meglio and colleagues [32] tested a protocol in which were given increasing, daily doses of raw hen's egg in order to reach a maximum dose of 25 ml of hen's egg in a 6-month period. The increase of the doses was mathematically calculated to achieve a constant 9% increment between doses in the first 80 days and 3.9% during the second part. The protocol foresaw the egg reintroduction in a home setting, and was performed in 10 patients with other 10 patients in the control group. Eight on 10 patients (80%) achieved a daily intake of the maximum doses, while only two in the control group (20%) achieved it. Tolerance to egg after a period of discontinuation of egg assumption was not assessed.

Another randomized open trial was performed by Dello Iacono and colleagues [33]. They tested a 6-month protocol of OIT in children with very severe hen's egg allergy (i.e., history of anaphylaxis or severe reactions after egg ingestion). The protocol consisted of a continuous increase of raw hen's egg from one drop to 40 ml in 180 days. The dose of hen's egg was doubled in hospital and slightly increased at home. None of the patients could eat a complete dose of 40 ml of egg without reactions, but 9/10 (90%) of children of the OIT group achieved partial desensitization ( $<40$  and  $\geq 10$  ml in a single dose) and 1 (10%) was able to eat only 5 ml. While performing the OIT, all the patients presented side effects, but no one presented a grade 5 reaction on the Sampson scale [34]. In the control group, nine patients didn't achieve any desensitization ( $p < 0.0001$ ). The outcomes of the OIT were not correlated with different factors such as age, gender, hen's egg white skin prick test weal diameter, hen's egg white-specific serum IgE levels.

In these last two studies, raw egg was used, and results seem to demonstrate that raw egg could be one of the best options, because proteins are kept intact and retain their allergenicity, although the safety could be not easy to assure. In particular, Dello Iacono and colleagues stated that if raw egg can be eaten, the diet can be safely liberalized. On the contrary, Escudero and colleagues [35], comparing the allergenicity of dehydrated egg white (DEW), a product that undergoes a double heat treatment (pasteurization and drying), with that of REW, determined that allergenicity of commercially available DEW is equivalent to REWs. *In vivo* and *in vitro* tests showed that processing of DEW does not affect the allergenicity of egg

proteins. Furthermore, DEW was demonstrated to be an effective and microbiologically safer source of allergen for the diagnosis of egg allergy, thus representing a more useful and risk-free option in egg oral immunotherapy.

Powdered pasteurized egg was used by Fuentes-Aparicio and colleagues [36], with a maintenance dose of 10 g (equivalent to one whole egg). They enrolled 72 patients with egg allergy and randomly assigned to OIT or elimination diet as a control group. Forty children (age ranging from 5 to 15 years) underwent an OIT; the control group included 32 patients (age from 4 to 15 years). Protocol duration was 13 weeks. Thirty-seven patients (92.5%) in the active group achieved desensitization to egg, versus 21.8% in the control group. The authors found statistically significant differences ( $p < 0.05$ ) for skin prick tests with powdered egg at various dilutions and IgG levels with EW after OIT, but specific IgE concentration did not change significantly. Tolerance acquisition was not assessed.

### Double-blind placebo-controlled trials

The first (and only), large, double-blind placebo-controlled clinical trial has been recently performed by Burks and colleagues, [37], and involved five research centers in the USA. The authors enrolled 55 children, aged 5–11 years: 40 received OIT with EW powder for 22 months and 15 were in the placebo group. The maximum dose of the maintenance was 2 g of EW powder, equivalent to one-third of the amount of proteins contained in one egg. This study aimed to evaluate the steps of desensitization during OIT and the achievement of tolerance after a period (8 weeks) of avoidance after the end of OIT. Oral food challenges were performed at 10 and 22 months. From 22nd to 24th months, immunotherapy was suspended and egg consumption was prohibited. Children who passed the challenge at 24 months were suggested to eat a regular amount of egg and were re-evaluated at 30 and 36 months. In the oral-immunotherapy group, the percentages of desensitized patients who passed the oral food challenge at 10 and 22 months were 55 and 75%, respectively, thus indicating that a longer duration of OIT is related with a higher rate of desensitization. After 2 months without any consumption of egg, only 28% (11 of 40) passed the oral food challenge and were considered to have 'sustained unresponsiveness' by the authors. These 11 patients passed the oral food challenge also at 30 and 36 months. The percentage of 28% is similar to what happens for the acquisition of natural tolerance, and it is not possible to assure that patients remitted for OIT or for their true natural history of egg allergy.

### Rush protocols

Trying to abbreviate the duration of the protocols and to improve the compliance of the patients, rush protocols have been performed, demonstrating to be capable to rapidly desensitize patients to allergens, and indirectly confirming the safety of fast immunotherapy for egg allergy (TABLE 3). In rush immunotherapy, higher doses are administered at 15- to 60-min intervals over a 1- to 3-day period, until the maintenance dose is achieved. In contrast to the conventional method, doses are

**Table 3. Rush protocols for egg allergy.**

| Study (year)                          | Protocol duration | Type of study      | Food vehicle | Age of patients | Patients (n) | Maintenance dosage                       | Results                     | Ref. |
|---------------------------------------|-------------------|--------------------|--------------|-----------------|--------------|------------------------------------------|-----------------------------|------|
| Itoh <i>et al.</i> (2010)             | 15 days           | Uncontrolled trial | PEW          | 7–12 years      | 6 OIT        | 7.5 g of egg proteins (whole egg)        | 100% DES                    | [40] |
| García Rodríguez <i>et al.</i> (2011) | 5–10 days         | Uncontrolled trial | Egg white    | 5–17 years      | 23 OIT       | 1.6 g of egg proteins                    | 86.9% DES                   | [39] |
| Ojeda <i>et al.</i> (2012)            | 37 days           | Uncontrolled trial | PstE         | 6–15 years      | 36 OIT       | 6.3 g of egg proteins (50 ml shaken egg) | 80% DES<br>3.2% partial DES | [41] |

DES: Desensitization; OIT: Oral immunotherapy; PEW: Powdered egg white; PstE: Pasteurized egg; RE: Raw egg.

doubled and given several times a day. The frequency of systemic reactions during rush immunotherapy is higher, but similar to conventional immunotherapy when premedication is used [38]. However, rush OIT is completely investigational and studies are limited, and in none of the studies the acquisition of tolerance was evaluated.

An ultra-rush protocol was performed by García Rodríguez and colleagues [39], with a duration of 5–10 days to reach the maximum dose of egg (2 g of EW powder). Twenty-three patients aged 5–17 years or older with IgE-mediated allergy to hen's egg were enrolled and after the ultra-rush desensitization protocol, were maintained on a regular egg intake. Twenty patients (86.9%) achieved a complete desensitization for a whole cooked egg, and 14 of these in 5 days. Severe reactions (i.e., anaphylaxis) were not reported, but other mild allergic reactions (i.e., oral or pharyngeal pruritus, abdominal pain, mild urticaria) were observed in all the patients. In the follow-up phase, egg was ingested without complications by all patients. At 6 months, after continuous regular intake of egg, skin prick test wheal size and specific IgE for EW were significantly diminished compared with the baseline.

Itoh and colleagues performed a rush protocol [40], which started from a dose of 1/10 of a previously assessed threshold for each patient, and reached in 15 days the amount of an entire scrambled egg (60 g). All the six patients enrolled could ingest the maximum dose at the end of the rush OIT and the authors concluded that a rush protocol (performed in a hospital setting for 5 days a week) would be more useful and more 'abandon free' than the longer protocols.

In 2012, Ojeda and colleagues [41] enrolled 36 children aged 6–15 years. The protocol had duration of 37 days, with daily increments of one- to twofold, and a final dose of 51 mg of pasteurized egg (6000 mg of egg proteins). Among the patients, 80.6% achieved complete desensitization to the maximum dose, equivalent to one raw hen's egg, 3.2% achieved partial desensitization and 16.2% could not ingest an acceptable dose of egg. Most of the latter patients had a positive baseline oral food challenge with low doses of boiled egg. Adverse reactions were common (74.2% of patients), with four subjects needing

adrenalin. Furthermore, more than half the reactions involved the gastrointestinal tract, with some of these having a delayed onset (even 9 h after the dose intake) and taking longer to resolve than those affecting other organs.

#### Heated egg: another source for OIT?

Extensively heated egg products have been found to be tolerated by a large number of patients with egg allergy, probably because extensive heating during baking reduces the allergenicity of the antigens, likely by denaturing the proteins and destroying structural non-linear epitopes [42]. After heating processes, thermolabile proteins (ovalbumin) may lose their allergenicity, while thermostable proteins (ovomucoid) do not undergo substantial modifications. In a murine model, Watanabe and colleagues [43] evidenced that heat treatment of EW improved allergic symptoms, inducing oral tolerance to ovalbumin. In particular after heating at 100°C for 5 min, egg consumption did not cause inflammation but induced tolerance, demonstrated by reduced T-cell responses and IgE levels. This 100°C heated egg demonstrated higher digestive stability and slower intestinal absorption.

In 2012, Lieberman and colleagues [44] described a series of 100 consecutive baked-egg challenges in their outpatient allergy clinic, confirming that the majority (66%) of egg-allergic children tolerate baked egg. There was no difference in terms of age, sex, history of asthma, anaphylaxis, atopic dermatitis or allergic rhinitis between patients who passed the challenge and patients who failed to pass. A recent work by Netting and colleagues in 2013 [45] indicates that up to 70% of children with egg allergy can eat baked egg in a cake or muffin without apparent reaction. Likewise, up to 75% of children can ingest baked goods containing cow's milk, and these children demonstrate IgE and IgG<sub>4</sub> profiles indicative of tolerance development.

It would be reasonable to think that these patients can develop a substantial tolerance to ovalbumin and that they are not allergic to ovomucoid, which is not modified by heat. In this case, prior to performing immunotherapy, it would be recommendable to evaluate specific protein IgE values through skin or blood test, currently available and not expensive (Prick

test, RAST, CAP system methodologies), and whose use has been reported to be reliable since the first report of Sampson and Ho [46], who compared the outcome of DBPCFCs with specific IgE values obtained by CAP System, and found they are generally comparable with those of skin prick tests in predicting symptomatic food hypersensitivity. In their experience after measuring the concentrations of food-specific IgE antibodies with the CAP System, it is possible to identify a subset of patients who are highly likely (>95%) to experience clinical reactions to egg, milk, peanut or fish, thus eliminating the need to perform DBPCFCs in a significant number of patients.

Reactivity to baked eggs has been investigated by Caubet and colleagues [47], who evaluated the specific (ovalbumin, ovomucoid) IgE/IgG<sub>4</sub> ratio, which was thought to add value to the measurement of absolute amounts of IgE for assessing the ongoing status of egg reactivity. Their data suggest that the IgE/IgG<sub>4</sub> ratio to ovalbumin and ovomucoid has important functional implications. Children reactive to baked egg have high IgE/IgG<sub>4</sub> ratio to ovalbumin or ovomucoid, whereas children tolerant to baked egg have low IgE/IgG<sub>4</sub> ratios. These results suggest that maybe some ovalbumin-specific IgE epitopes can react with parts of ovalbumin not degraded by heat.

Ingestion of baked egg appears to accelerate the development of tolerance to natural/raw egg. In 2008, Lemon-Mulé and colleagues [48] reported their results regarding the immunologic changes associated with a continued ingestion of heated egg in egg-allergic patients and demonstrated that the majority of subjects with egg allergy were tolerant to heated egg. Furthermore, the prolonged ingestion of heated egg was associated with decreased skin test wheal diameter and ovalbumin-specific IgE levels. These patients also presented increased ovalbumin- and ovomucoid-specific IgG<sub>4</sub> levels. Also the experience of Leonard and colleagues [49] aimed to characterize immunologic changes associated with ingestion of baked egg and evaluate the role that baked egg (one- to three-times per day) diet plays in the development of tolerance to regular egg. Seventy-nine were instructed to incorporate all forms of egg into their diet and were encouraged to do so at least twice a week, and among these at the end of the protocol, 42 (53%) were able to eat raw egg, 28 (36%) could eat only baked egg, 9 (11%) weren't able to ingest any form of egg. In the control group (47 patients), 28% were able to eat raw egg, 13% baked egg and 59% couldn't eat egg. Subjects in the OIT group were 20.9-times more likely to eat baked egg ( $p < 0.0001$ ) and 18.3-times more likely to eat regular egg ( $p < 0.0001$ ) than the comparison group. It is important to underline that of 23 initially baked egg-reactive subjects, 14 (61%) subsequently could consume baked egg and 6 (26%) regular egg. Among this baked egg-reactive group, subjects with persistent reactivity to baked egg had higher median baseline EW-specific IgE levels than those who subsequently could eat baked egg. Also in this group of subjects ingesting baked egg, ovalbumin- and ovomucoid-specific IgE/IgG<sub>4</sub> decreased significantly, thus demonstrating that regular baked egg assumption can improve tolerance to both thermolabile and thermostable proteins.

In contrast to these positive results, a report by Tey and colleagues [50] in 2012 highlighted that frequent consumption (once/week) of baked egg was not associated with a different rate of decline in egg skin prick test size in egg-allergic children. Although no differences were seen in the occurrence of side effects, the authors concluded that it would be reasonable to consider liberalizing baked egg in the diet of egg-allergic children, but no 'immunotherapeutic' effect was reported. In another study, Turner and colleagues [51] performed challenges to egg-allergic patients presenting in their clinic, with incremental dosages to a total of one baked muffin containing 1 g of egg protein, in order to evaluate reactivity to baked egg. Although 64% of patients passed the challenge, 36% reacted and 14% experienced anaphylaxis. For this reason, they stated that challenge with baked/heated egg should be performed under medical supervision.

### Safety of egg immunotherapy

OIT safety was assessed in all the studies. Common symptoms included cutaneous reactions or gastrointestinal symptoms, especially in subjects with higher IgE levels, or with past episodes of severe reactions.

Studies in which high-risk patients were enrolled were the ones in which side effects were more common, in particular in the study by Dello Iacono and colleagues [33], the frequency of side effects was 100%; in this case, daily premedication with antihistamines is highly recommended. In slower protocols performed in patients with mild-to-moderate symptoms of egg allergy, severe reactions were less common and the occurrence of minor symptoms was lower. However in some slow protocols, episodes of anaphylaxis or severe reactions were reported [36]. Side effects occurred both in OIT and control groups. In the study by Burks and colleagues [37], adverse events, most of which were oral or pharyngeal, were associated with 25.0% of doses of OIT and 3.9% of doses of placebo. In the OIT group, 78% of children had oral or pharyngeal adverse events, as compared with 20% of those in the placebo group. After 10 months, the rate of symptoms in the oral-immunotherapy group decreased to 8.3%. Higher doses of allergens were associated with higher risk of side effects. Among the 72 patients of OIT protocol of Fuentes-Aparicio and colleagues (maintenance dose 10 g of egg proteins), 21 presented allergic reactions, with severe reaction in 13 cases [36].

Comparing the side effects of rush versus slow protocols, a higher frequency was found in rush protocols, ranging from 100% of patients in the study by Itoh and colleagues [40], 78.3% in the study by García Rodríguez [39] and 74.2% in Ojeda and colleagues [41]. In this last study, in four occasions, symptoms involved more than three organs and required auto-injectable adrenalin use.

Tolerability is higher in the protocols using heated or baked egg [48,49], in which the occurrence of side effects is limited.

### Conclusions

In the first 15 years of egg OIT, different protocols have been adopted, with different amount of proteins, food preparations,

duration, number and classes of patients treated. Although at present egg OIT is not a standard, acceptable clinical practice and is considered as investigational by many experts in the field, these experimental studies deserve to open a new frontier in immunotherapy for egg allergy.

Through the revision of the protocols, it seems that a large percentage of patients respond positively to OIT. The percentage of desensitized patients varies from 25 to 100%: patients with less severe forms of allergy and lower levels of IgE tend to respond better to OIT. Confirming this, the study by Dello Iacono and colleagues [33], performed in patients with history of anaphylaxis, showed a low amount of desensitization. It is still a matter of debate if the tolerance acquisition observed during OIT protocols should be related to the natural history of the disease or is induced by OIT. The low percentage (28%) of tolerant patients after OIT found in the only double-blind placebo-controlled study [27] seems too similar to what usually happens in untreated patients, and thus those reported by Buchanan and colleagues [26] and Staden and colleagues [30] (28.6 and 36%, respectively). Higher results in terms of tolerance acquisition were reported only in the study by Vickery and colleagues [28], in which 6/8 (75%) patients developed tolerance to egg; however, it must be underlined that the sample of treated patients was too small.

The amount of egg proteins does not seem to be related to better results, and this could be described by the fact that most of the food allergy are not 'dose-dependent', and by the fact that both small and big amounts of allergen can determinate the immunological processes of desensitization or tolerance acquisition [20–22]. However, in all the studies examined, the maintenance dose was at least 300 mg/day. Together with this, the consumption of raw, powdered or heated egg does not lead to higher quantity of responders, probably because the heat processes do not denature at all eggs protein, and some (more) allergenic epitopes may remain unaltered.

Concerning the duration of OIT, no difference in success rates between rush and slow protocols was observed, even if the published rush protocols are still today small and uncontrolled studies. Rush protocols should be preferred if performed in hospital settings, while slower, clusters protocols are more suitable to be performed at home, because of a significant lower occurrence of side effects. Further attempts to standardize the

procedures of oral desensitization are expected, and in particular head-to-head studies using otherwise similar protocols are really required to be able to make conclusive statements about rush versus slow protocols for OIT.

Future OIT studies should evaluate the acquisition of tolerance and include placebo and active groups to control natural resolution of allergy, because egg allergy is associated with high rates of spontaneous resolution. Meta-analyses of OIT studies evaluating tolerance as the main outcome are warranted, once additional randomized controlled trial will be performed.

### Expert commentary

As seen above, egg immunotherapy is a field in rapid evolution and current status of the research indicates that different protocols, different dosing, different timing lead, in most of the cases, to the acquisition of desensitization. The information regarding the tolerance acquisition is still modest, and in the main studies the rate of tolerance acquisition after OIT is comparable with the natural history of the disease. Only a few studies have included high-risk patients, in which immunotherapy seems more harmful but can lead to the acquisition of a partial tolerance. Food vehicles selection seem to be important for the protocol's modality, but not for the final results, because the rates of success are comparable.

### Five-year view

An increasing number of protocols for egg immunotherapy is expected in the next 5 years. Evaluation of tolerance through specific immunomarkers (CD4<sup>+</sup> hypoproliferative subsets of cells, CD63<sup>+</sup> cells) will be one of the research goals of the future. A 'pre-protocol identification' of the immune system of the patients is also expected, which will allow the allergologist to select the 'good patient' in whom immunotherapy will be efficacious.

### Financial & competing interests disclosure

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

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### Key issues

- Egg allergy affects 1–3% of the pediatric population in the Western countries and is a potentially lethal disease.
- Egg avoidance is difficult to practice because of the presence of unnoticed quantity of egg in foods.
- In the last 15 years, an increasing number of protocols for desensitization have been performed, with different dosing of allergens and timing.
- Rush protocols demonstrate safety and efficacy comparable with slow protocols.
- Desensitization rate is higher than 80% in most of the published protocol.
- Results for high-risk patients (i.e., anaphylaxis-history positive) are worse, although a partial desensitization is reached in most of them.
- Although the evaluation of tolerance acquisition has not been always performed, results in this way seem to be similar to what happens in the natural history of egg allergy.
- In the future, tolerance acquisition could be demonstrated by specific immunomarkers (CD4<sup>+</sup> hypoproliferative subsets of cells, CD63<sup>+</sup> basophils activation) and a positive threshold for these values remains to be determined as being predictive for tolerance acquisition.

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