Oral Immunotherapy

How effective is oral immunotherapy for food allergies and can it already be applied in clinic?

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Contents

Abbreviations

OIT	Oral immunotherapy
Ig	Immunoglobulin
MeSH	Medical Subject Headings
DBPCFC	Double Blind Placebo Controlled Food Challenge
SBPCFC	Single Blind Placebo Controlled Food Challenge
SPT	Skin Prick Test
CM	Cow Milk
HE	Hen's Egg
CD	Cumulative dose
MRP	Modified Rush Phase
BP	Build-up Phase
MP	Maintenance Phase
ED	Elimination diet
M cells	Microfold cells
DC	Dendritic Cell
APC	Antigen-Presenting Cell
IL	Interleukin
Th-cell	T-Helper cell

Abstract

In development countries the number of people suffering from food allergies is on the rise. Currently, the main hope for patients is to grow out their allergy while on an avoidance diet. In this review we focus on oral immunotherapy (OIT), a potential treatment for food allergies. After the introduction, the research method is described. Subsequently, the general principle of OIT is explained. The results describe the main points of each article which are included in this review. The main points are described in the context of efficacy and safety. The description of the main points is followed by a discussion of these points. Of the 274 patients, enrolled in twelve clinical trails included in this review, 181 patients (66%) full desensitized after treatment. Control groups were included to examine if desensitization and/or tolerance are/is induced by OIT or by natural outgrow. Some studies performed an elimination diet after treatment to examine if the effect of OIT is persistent or transient. The effect of OIT is significant positive straight after treatment compared to the control groups. This effect finished after an elimination diet and this indicates that OIT thus not induce tolerance and that OIT contribute towards desensitization. However, there are some disadvantages. The results are supported only by a limited amount of studies and the general conclusions are debatable because there is much variation between studies in protocols (duration of therapy, dose increase scheme, way to establish allergies) and characteristics of patients enrolled in the trails (mean age, multiple food allergies, severity of allergy). Furthermore, some studies have not included control groups, did not performing an elimination diet after OIT, and did not enrolling a reliable number of patients in there studies. There is needed more knowledge for we can apply this treatment in clinics. In addition, it is difficult to say if OIT therapy is safe enough to apply in clinic. To collect comparable information about the safety of this therapy it is advisable to use a reliable method to establish the severity of side-effects. Furthermore, it is important to examine the effect of OIT on different groups with discrepancies in age, kind of allergy, multiple allergies etc. Thus, more research is needed.

1. Introduction

In developed countries the number of people suffering from food allergies is on the rise. The prevalence of reported food allergy is increased by 18% from 1997 to 2007 (Branum et al. 2009). About 2-3% of the adults and up to 6% of the children suffer from food allergy (Pereira et al. 2005, Eigenmann et al. 2003). Current management of food allergy includes avoidance of the allergenic antigen and treatment with numerous medicines (e.g., epinephrine, antihistamine) if someone accidentally has ingested a critical amount of allergen. Each year, a significant proportion of children do accidental ingest a critical amount of allergen (Clark et al 2008). This is the reasons why quality of life is reduced in patients with food allergy (Flokstra-de Blok et al. 2009). Flokstra-de Blok and colleagues compared health-related quality of life of food allergic patients with that of the general population. They find that food allergic patients report more pain, have an inferior overall health, have more limitations in social activities and are less vitality than individuals from the general population. In addition, it is demonstrated that children with milk allergy or multiple food allergies are at greater risk of growth problems by inadequate nutrient intake (Christie et al. 2002). Fortunately, it is possible, especially in young children, to grow out the allergy. Presently, this is the main hope for patients while on an avoidance diet. Furthermore, education about emergency measures in case of accidental food ingestion is important (Sicherer et al. 2006).

Recently much research is done to contrive a solution for suffering from food allergies. Various types of therapies are applied and will be applied to release the allergic patients of their allergy. For instance Leung and colleagues (2003) investigated the effect of anti-immunoglobulin E (IgE) therapy on patients with peanut allergy. They concluded that IgE antibodies protected at least a subset of patients by increasing their threshold dose for allergenic responses. Unfortunately, anti-IgE therapy is expensive and will not change the natural history of allergic diseases. Furthermore, sublingual immunotherapy could be a potential therapy. One of the first studies, with sublingual immunotherapy, is done by Oppenheimer et al. (1992) and Nelson et al. (1997). This research group applied subcutaneous immunotherapy in patients with peanut allergy. Regrettably, the treatment induced systemic reactions and an unacceptable high rate of severe side-effects and therefore this therapy could not be recommended. By these side-effects today's focus lies in oral immunotherapy (OIT). Patients who underwent oral immunotherapy ingest gradually increasing amounts of allergens to induce desensitization and/or tolerance. Desensitization is defined as a change in the threshold of ingested food antigen needed to cause allergic symptoms. Tolerance is a long-term immunologic change associated with the persisting to ingest food without symptoms and without ongoing therapy (Jones et al. 2009). In this paper we focus on oral immunotherapy.

While there have been performed many clinical studies utilizing oral immunotherapy, a dearth of research has considered to provide an overview of such findings to draw more generalized findings. To address this gap, this paper investigates the contribution and findings of A-ranked papers, published up to now to facilitate the creation of a broadening of our knowledge.

Some researchers have investigated this question before (Niggeman et al. 2006, Beyer et al. 2008, Sopo et al. 2009, Skripak et al. 2009, Nowak-Wegrzyn et al. 2010). Some of these reviews are written several years ago and recently more than a few clinical studies are published (Niggeman et al. 2006, Beyer et al. 2008). One other study focuses only on milk allergies (Skripak et al. 2009). Furthermore, all studies suffer from the limitation of not including critical information concerning the methodology applied. In addition, it is essential that the introduction of a new therapy is guided by a substantial amount of studies.

The goals of this paper are threefold:

(i) to create a better understanding of how current findings of studies regarding oral immunotherapy can be utilized in clinics;
(ii) to articulate the possible research gaps to stimulate further research into oral immunotherapy;
(iii) to examine what is know about the mechanism of OIT.

More specifically, our research question is as follows: 'How effective is oral immunotherapy for food allergies, which mechanism lies behind OIT and can it already applied in clinic?'

The efficacy of OIT will be described briefly in the context of long-term tolerance versus transient desensitization and furthermore the safety of OIT will examined. However, first it is crucial to provide you with an overview of the methodology and the principles of OIT. Finally, the mechanism behind oral tolerance, allergies and OIT will be discussed.

2. Methods

During this research it is noticed that there is no general name for the treatment where patients administrate gradually increasing oral dosages of the offending food to finally desensitize. The name 'oral immunotherapy' is most often utilized. Thereafter 'specific oral tolerance induction' is quite admired. Besides these two names, 'oral desensitization' and 'oral hyposensitization' are used as well. In this review the term 'oral immunotherapy' is applied to indicate the performed therapy.

In some studies also sublingual immunotherapy with food is referred to as 'oral immunotherapy'. Sublingual and oral immunotherapies are both promising therapies used to solve food allergy involving patients who ingest steadily increasing dosages of the offending food. However these therapies are dissimilar in some respects. For instance, they differ in the way of food intake. With sublingual immunotherapy the patients have to keep the allergen in their mouth for several minutes before they swallow the food. With oral immunotherapy patients discharge the allergic food immediately. Most often sublingual immunotherapy is also performed with smaller amount of the allergic protein and therefore it is not reliable to include both forms of oral immunotherapy.

Relevant publications were sought through computerized searches in Pubmed and Embase using the following medical subject headings (MeSH): oral immunotherapy, oral desensitization, oral hyposensitization, specific oral tolerance induction, oral administration, and food hypersensitivity in combination with food allergy, milk allergy, peanut allergy and egg allergy. Furthermore, the abstracts of the EAACI pediatric Allergy and Asthma Meeting of November 2009 were consulted. This journal contains the abstracts of the most recent ongoing clinical trials. Searches were performed in February 2010, April 2010 and May 2010.

There are a number of criteria established and applied to ensure that no more than articles with reliable information were included in this study. In this review, most preferred were randomized double blind placebo controlled trials. Randomized controlled trials are the best way to verify the efficacy of the therapy, since it is feasible that patients could grow out their allergy. Including a control or placebo group it is achievable to eliminate the effect of outgrowing the allergy and establish the real influence of OIT. A double blind placebo controlled food challenge (DBPCFC) is the best way to diagnose food allergy, because the outcome is the safest and the most consistent. Patients may be unnecessarily diagnosed being severely food allergic without DBPCFC (Vlieg-Boerstra et al. 2008).

Only three randomized DBPCFC trails are available nowadays to establish the presence of food allergy before OIT (Skripak et al. 2008, Caminiti et al. 2009, Staden et al. 2007). Morisset et al. (2007) published also a randomized study but used a singe blind placebo controlled food challenge (SBPCFC) to establish the presence of food allergy. Due to this small number of studies we decided to include

also studies which performed DBPCFC to settle the diagnose of food allergy and did not include a control group (Meglio et al. 2004, Aragones et al. 2007, Meglio et al. 2008, Staden et al. 2008, Clark et al. 2009, Itoh et al. 2010). In addition, studies which used DBPCFC to establish if OIT was effective (Buchanan et al. 2007) and studies who included a control or placebo group (Patriarca et al. 2003) or a combination of these (Rolinck-Werninghaus et al. 2005, Longo et al. 2008) were included. Though the number of patients enrolled in these studies was considered low, they were included in our review.

In total, twelve papers were included. The paper of Hofmann and colleagues (2009) was excluded because the allergy diagnose for peanut was established, based on the presence of specific IgE to peanut, with a skin prick test (SPT) and a CAP-FEIA. In addition, they do not include a control our placebo group. This also applies to the article of Jones et al. (2009).

3. Principle of Oral Immunotherapy

There is much variation in the approaches of OIT and so far no uniform protocol has been developed. What applies to all protocols is that a gradually increasing amount of a known allergic food is delivered via the gastrointestinal route to finally desensitize patients. Protocols differ in: starting dose, up dosing schedule, and maintenance dose (Table 1).

The first dose of the allergic food given by oral immunotherapy is always very low (Table 1). Sometimes, the starting dosage is derived from DBPCFC. Patients with milk allergies get almost in nearly all cases diluted milk first followed by whole milk (e.g., Caminiti et al. 2010, Longo et al. 2008). Patients with egg and peanut allergies generally get powdered food mixed with a food vesicle (e.g., Clark et al. 2008, Itoh et al. 2010).

In about 50% of all trials a rush phase is performed (Table 1). A rush phase lasts mostly one day, but some studies used 5-10 days for this phase. When the rush phase last one day, mostly the dosage will double every 30 minutes. The rush phase takes place in hospital and the goal of this phase is a rapidly build-up of dose threshold for safety and time reduction.
The rush phase is followed by a phase where the doses slowly but gradually increase (Table 1). When no rush phase is performed, the protocol starts with this phase. In this paper we call this phase 'the build-up phase'. In some cases the dose of the allergic food increases daily with a small amount of the allergic food, mostly the dosage increase every 7-14 days. Dose increase takes place in hospital or at home. The duration of the build-up phase depends on how fast doses can increase without moderate or severe allergic symptoms.
The maintenance phase is followed by the build-up phase (Table 1). During the maintenance phase the dosage of the allergic food will not increase. Various studies differ in the height of the maintenance dose (Table 1). Patients continue to ingest the intent dose or the highest feasible dose every day or several times a week. This phase takes several months till years.

During all phases patients are monitored several times for safety and data collection. Patients are often provided with self-injectable epinephrine and/or antihistamines. Especially in studies were up dosing takes place at home (e.g., Staden et al. 2007, Longo et al. 2008, Clark et al. 2008). Furthermore, in a few studies a dedicated telephone number is available 24 hours a day and family of the patients are trained in medical treatment.

At the end of the protocol it is intended that patients could eat usual quantities of the antigen in their diet. This is not always feasible and in these cases it is pleasant when the patient is able to ingest a higher amount of the allergen than before (Table 1). In these cases danger associated with accidental exposure to small amounts of the food might be reduce and quality of life might be increase.

Oral immunotherapy is applied in patients allergic for various foods. The articles, utilized for this review, in general describe oral immunotherapy for milk, egg, cod and peanut allergies.

4. Results

4.1. Efficacy of OIT

The first study published and included in this review was published in 2003 by Patriarca et al. (2003). Patriarca et al. included 58 patients (age between three and 55 years) with allergies to various allergens. In table 1, only the patients with milk, egg and fish allergy are included, because these groups of patients were clearly described in the article in contrast to the patient groups with other allergies. In addition, the groups of patients with other allergies were small (one to three patients each group). Patriarca et al. enrolled adults in their clinical trial. This is obvious because no other studies described and included in this review have done this. However, also in this article 60% of the patients were children aged less than sixteen. Six of the 59 patients included showed a positive clinical reaction to more than one food allergen. These six persons underwent thirteen treatments. In total, 66 treatments were performed. No other study included in this review desensitized for more than one allergen. Finally, 83.3% of the treatments were completed successful. They did not find any difference between adults and children and no significant difference in the outcome of the therapy between various allergens.

The second study included, is the study of Meglio and colleagues (2004). They performed oral immunotherapy on cow's milk (CM) allergic children, using DBPCFC to establish the diagnosis. In this study 21 children (five-ten yr.) underwent oral immunotherapy for their milk allergy. Because of the length of the protocol Meglio et al. chose an entry age of five for admittance the trail. The higher the age at inclusion, the lower the probability of becoming naturally tolerant to cow milk. After a build-up (six-eight months) and maintenance phase (length not specified) of the patients were intended to ingest 200 ml milk daily. At study conclusion 71.4% were able to drink 200 ml and 14.3% could tolerate a lower dosage.

In 2008 Meglio et al. (2008) published a follow-up study with twenty of the 21 children of their first study. The only dropout belonged to the group who reached totally desensitization previously. During the past four years children had to drink cow's milk daily, but were free in the amount. All children underwent a SPT (skin prick test). Furthermore total IgE and specific IgE (casein and α-lactoalbumin) were determined to examine if tolerance was lost or persistence. After four year and eight months, 70% of the children totally or partially tolerated milk. Six out of twenty children could not tolerate CM at follow-up. The three patients who could no tolerate CM in the previous study are included in this group. Compared to the previous study (85.7% totally or partially tolerated CM). Meglio et al. indicate a persistent oral desensitization effect, but they also indicate that desensitization induced food tolerance can decline. One child interrupted the consumption of cow's milk and experiencing urticaria and asthma when she continued the diet after one month. Furthermore, Meglio and colleagues stress the importance of partial outcome, which refers to patients tolerating a higher amount of allergen than before treatment, but did not reach the maintenance dose. This might reduces the risk of severe reactions after accidental or unnoticed ingestion of a small amount of CM. This is the longest follow-up (four years and eight months) published up to now. It is regrettable that Meglio et al. did not perform a control group in parallel. Consequently, it is difficult to conclude whether tolerance was gained through immunotherapy or that the process was natural.

In 2005 Rolinck et al. designed a protocol including a time interval of eight weeks to demonstrate if oral tolerance induction is persistent or transient. Only three articles included in this review have described the effect of a time interval (Rolinck et al. 2005, Staden et al. 2007, Buchanan et al. 2007). A limitation of this study is the number of patients (n=3) included in the trial. After a build-up phase (37 weeks) and a maintenance phase (27 weeks) all patients could tolerate cow's milk (n=1) and hens eggs (n=2). After receiving a strict elimination diet, a diet without consuming the allergen at all, for two months none of the patients were still tolerant. Rolinck and colleagues hypothesize that tolerance

induced by oral desensitization is not a persistent nature, but raise the threshold dose necessary to elicit allergic symptoms.

Subsequently Morisset (2007) reported the induction of desensitization in 88.9% of the children with milk allergy (n=27) and 69% of the children with egg allergy (n=49). Instead of DBPCFC this group used SBPCFC to establish the diagnose food allergy and establish the effect of OIT. They also included an untreated control group and therefore this is the first published randomized study considering oral desensitization in food allergic children. The authors explain why it is important to perform clinical trials in this way. They argue: 'Only the difference in recovery rate of the two groups after six months will demonstrate any benefit of oral desensitization'.

Aragonés (2007) reported the induction of desensitization in four patients with cow's milk allergy. These four children could tolerate 200 ml cow's milk after a modified rush phase of five days. After three years of follow-up the four patients are still taking cow's milk with good tolerance. During these years the patients had to drink cow's milk every day. Resembling to Meglio et al. (2004, 2008) no control group was examined in parallel and therefore it is difficult to conclude whether tolerance was gained through immunotherapy or that the process was natural. Subsequently, the patients included in this study were very young (a two years-old boy, a two years-old girl, a nineteen months girl and a five years-old boy). According Host and colleagues (2002) approximately 90% of the children recover cow's milk allergy within three years. This indicates that probably the gained tolerance, from 75% of the cases, is induced naturally.

A randomized double blind placebo controlled food challenge including a large group of children (OIT group: n=25, control group: n=20) with cow's milk and hen egg allergy was published by Staden (2007). After a build-up phase (~seven months) and maintenance phase (~nine months) 48% were complete tolerant and 16% were partial tolerant to milk and egg. After these phases the patients of Staden et al. underwent an elimination diet. Thereafter they performed a DBPCFC (not immediate after OIT) to obtain reliable results. Thirty-six percent of the children still remain tolerant. Looking at the efficacy rate no difference is seen between the OIT group (36% tolerant after elimination diet) and the untreated group (35%). This indicates that oral immunotherapy did not have a persistent effect and thus does not change the natural course of cow's milk and hen's egg allergy in young children. However, by including children who reached partial tolerance the efficacy rate of the OIT group increased. This group included patients who required a regular intake of CM to maintain tolerance or those who can tolerate a dosage lower than the standard maximum dose. Staden et al. concludes that OIT can possibly induce clinical tolerance and they indicate that clinical tolerance could be favorable for children with a severe allergy who are at higher risk for severe allergic reactions at accidental ingestion of an allergen.

Buchanan (2007) induced oral desensitization to egg in four out of seven allergic patients. Additionally, three out of seven patients became partial tolerant. Patients with a negative outcome (n=4) of the first DBPCFC after OIT underwent a second DBPCFC after an elimination diet, a diet without consuming the allergic allergen for three-four months. After this interval two out of four patients were still tolerant to egg. This study did not include a control group and the children enrolled this study were very young. Similar to previous described studies it is not clear whether persistent tolerance is induced by OIT or if it is induced naturally. Although tolerance could be induced naturally, the time interval was so short that it is questionable whether tolerance can be induced naturally in this time frame. In addition, the OIT group was very small.

Longo et al. (2008) shows, for the first time, that OIT can be achieved in patients affected by severe food allergy due to including children with a positive outcome of a DBPCFC with a very low threshold. Children were included in the study if they had symptoms during the lowest dose at the DBPCFC (up to 8 mL of diluted formula or 0.8 ml of whole milk). This is an extremely low threshold compared to Caminiti et al. (100 ml whole milk) and Rolinck et al. (150 ml whole milk). In most studies, children affected by such a severe food allergy were excluded for safety. Longo et al. performed a rush phase of ten days. Thereafter doses were increased every two weeks for an undefined

time followed by a maintenance phase continued for an undefined time. Finally, the group of Longo induced desensitization in eleven out of thirty patients (able to drink 150 ml milk) and partial desensitization in sixteen out of thirty patients (able to drink 5-150 ml). The effect of OIT was significant effect compared with a control group of thirty patients. The existence of OIT and the consequences of a time interval were not evaluated.

Clark and colleagues (2008) concluded, after a build-up phase (duration not specified) and a maintenance phase (duration 6 weeks) that a substantial improvement in the tolerated dose occurred in all subjects (n=4). Up to now this is the only study, including peanut allergic children, which diagnosed peanut allergy with DBPCFC before treatment. Unfortunately, they did not take in a control group. Moreover, the patient group was very small.

Skripak and colleagues (2009) provide evidence that orally administered immunotherapy can help patients with milk allergy. Due to a rush phase of one day, a build-up phase of three to twenty-three weeks and a maintenance phase of thirteen weeks, they desensitized 92% children (n=13) with cow's milk allergy. This percentage is relative higher than described in previous published studies (e.g., Patriarca et al. 2003, Morisset et al. 2007, Staden et al. 2008). Possibly, this is due to the fact that the amount of food allergen ingest in the maintenance phase was much lower (15 ml Skripak versus 200 ml Meglio) than in other studies. The children enrolled in the study of Skripak could reach the maintenance dose more easily by this low threshold. However, the conclusion of Skripak et al. is supported by the results of a control group, were zero out of seven became tolerant. Skripak et al. did not invest if the effect of OIT is persistent or transient, because they did not perform a time interval after OIT or a follow-up for a long time.

Recently, Itoh et al. (2010) published a unique protocol for OIT similar to the protocol published by Staden et al. (2007). The protocol started with a rush phase of twelve days. Five days a week patients (n=6) with an egg allergy stayed in hospital during the rush phase. Every Saturday and Sunday the patients went home and there the patients ingested the highest tolerated dose up to then, 3 times a day within at least one hour interval. The starting dose of this protocol was one tenth of the threshold dose established with DBPCFC. Subsequently, the rush phase was followed by a maintenance phase of nine-twelve months. During this phase patients had to eat one whole egg twice a week. After sixteen-21 months all patients tolerated this dose without symptoms. Itoh and colleagues showed that a rush OIT can induce tolerance in school-age patients with severe food allergy in a few weeks. However, this was an uncontrolled study. Itoh et al stated: 'the effect of this rush OIT would be evident because they establish the diagnose by DBPCFC before treatment and it would be unlikely that the allergic condition would vanish naturally in a few weeks'.

The last study described in this review is the study of Caminiti et al. (2010). Caminiti and colleagues enrolled thirteen patients in their study. Six of the thirteen patients underwent a double-blind placebo-controlled challenge (including the control group, n=3) and seven children underwent the SOTI procedure in an open fashion. They performed a build-up phase during thirteen weeks and a maintenance phase during four months. After treatment a DBPCFC was performed for 30% children of the OIT group (n=10) and 100% for the children of the control group. 70% of the patients who underwent the OIT were tolerant after a protocol of four months. 10% was partial tolerant to cow's milk. This result was significantly effect compared to the control group where 0% became tolerant of partial tolerant. The results achieved in this study are comparable in terms of clinical outcome and failures to other studies using long protocols (e.g., Meglio et al. 2004). Caminiti did not investigate the long-term effect of OIT and the effect of a food intake interval of the food the patient is allergic for.

Table 1: Overview of final results in terms of efficacy from oral immunotherapy trials: The table summarized the characterizes of all included papers in terms of efficacy in the context of long-term tolerance versus transient desensitization.

Author, year	Food	Age in months	DBPCFC range to establish allergy before OIT	Starting dose	OIT protocol	Duration of BP phase	Duration of MP phase	MP dose	Instrument to establish the result of OIT	Full desensitization (responder + regular intake)	Partial desensitization (tolerated lower dosage)	Tolerance success rate (tolerant after interval)
Patriarca et al. 2003	Milk (n=29) egg (n=15) cod (n=11)	36-660	Range: 0.01-30 ml CD: 46.6 ml (milk, egg) Range: 0.001-20g CD: 38.6 (cod)	5 ml (milk) 0.5 ml (shaken egg) 0.000033 mg (cod)	**BP:** Dose increase every three days **MP:** All patients were asked to eat the allergic food at least twice a week. → some patients underwent more than one protocol	Milk: 3-12 months Egg: 3-8 months Cod: 4-10 months	Not specified	120 mL milk 1 egg 160 mg boiled cod	None	Milk: 19/29 (65.5%) Egg: 11/15 (73.3%) Cod: 8/11 (72.7%) *Control group: 0/16 (0%)*	Milk: 5/29 (17.2%) Egg: 2/15 (13.3%) Cod: 2/11 *(18.2%)* *Control group: 0/16 (0%)*	Not tested
Meglio et al. 2004	Milk (n=21)	63-122	Range: 0.5-162.5 ml CD: 230 ml (milk)	0.0017 ml (milk)	**BP: Dose d**oubled every 7 days until day 70, Than the dose is doubled every 16 days until a dose of 200 ml is reached	6-8 months	Not specified	200 ml milk daily	None	15/21 (71,4%)	3/21 (14.3%)	Not tested
Meglio et al. 2008 (Follow up of Meglio et al. 2004)	Milk (n=20)	63-122 (4-5 yr later)	Not applicable	Not applicable	**P**atients were advised not to discontinue free daily intake for 44 months (mean)	Not applicable	Not applicable	200 ml milk daily	none	13/20 (65%)	1/20 (5%)	not tested
Rolinck et al. 2005	milk (n=1), egg (n=2)	58.8-154.8	Range: ? - 150 ml CD: ? (milk) Range: ? CD: ? (egg)	0.0006 ml (milk) 0.0029 mg (egg)	**BP:** dose increase each day until a maximum dose of 250 ml CM or 4.5 g egg protein **MP:** daily intake milk or egg + deliberate intake.	37 weeks (milk) 41-52 weeks (egg)	27 weeks (milk) 39 weeks (egg)	100 ml milk daily 71.4 mg egg daily	None	Milk: 2/2(100%) Egg: 1/1 (100%)	0/3(0%)	After two months: 0/2(0%)
Morisset et al. 2007	Milk (n=27) Egg (n=49)	12-96	Range: 0.1- 40 ml CD: 60 ml (milk) * Range: 5 -700 mg CD: 965 (Raw egg white) #	1 ml (whole pasteurized milk) 1g yolk (egg)	**BP for milk:** dose increase each day in the first week. Dose increase every week in the second/ third and fourth week. In the fifth starts the introduction food containing the allergic protein. **BP for egg:** dose increase every week until the fourth	7 weeks (milk) 3 months (egg)	6 months (milk and egg)	Not applicable	SBPCFC	Milk: 24/27(88.9) Egg: 34/49 (69%) *Control group milk: 18 /30 (69.4%)* *Control group egg: 25/49*	Milk: 0/27 (0%) Egg: 0/49 (0%) *Control group milk: 0/30 (0%)* *Control*	Not tested

					week. In the second month starts the introduction of food containing the allergic protein. **MP for egg and milk:** daily intake milk, egg or food containing the allergic protein.					*(51.4%)*	*group egg: 0/49 (0%)*	
Aragones et al. 2007	milk (n=4)	19-60	Range: not specified CD: not specified	0.01 ml (milk)	**MRP:** 5 days, 2-5 doses per day, dose is doubled every hour until 200 ml of CM is reached **MP:** continue drinking milk each day	Not specified	36 months	200 ml CM daily	none	4/4(100%)	0/4(0%)	not tested
Staden et al. 2007	Milk (n=14), egg (n=11)	7.2-154.8	Range:? -94.3 mg CD: 136.3 mg (milk) Range:? -131.4 mg CD: 177.1 mg (egg)	0.0057 mg (milk) 0.00017 mg (egg)	**BP:** doses increased according individual tolerance **MP:** daily intake of maintenance dose + deliberate intake.	7 months (medium)	9 months (medium)	100 ml CM daily ¼ egg daily	DBPCFC after a two months elimination diet	12/25(48%) (Not clear if these children milk or egg allergic) *Control group: 0/20(0%)*	4/25(16%) *Control group: 0/20(0%)*	After tow months elimination diet: 9/25(36%) *Control group: 7/20(35%)*
Buchanan et al. 2007	Egg (n=7)	14-84	not applicable	0.1 mg egg white powder	**MRP**: one day, dose doubled every 30 minutes until highest tolerated single dose was determined **BP**: 1 dose daily, dose increase every two weeks. **MP:** daily intake of maintenance dose.	Not specified	Not specified	8.57 mg egg daily	DBPCFC	4/7(57%)	3/7(43%)	2/4(29%)
Longo et al. 2008	Milk (n=30)	60-204	Range: 0.025 – 8 ml CD: 15 ml (milk)	0.5 ml (milk)	**MRP:** 10 days, several doses daily until subjects reach 20 ml **BP:** dose increase every second day with 1 ml **MP:** maintenance dose + deliberate intake.	Not specified	Not specified	150 ml CM daily	DBPCFC (the control group) Open food challenge	11/30(36%) *Control group: 0/30 (0%)*	16/30 (54%) *Control group: 0/30 (0%)*	not tested
Clark et al. 2008	Peanut (n=4)	108-156	Range: 1-100 mg CD: 256 mg (peanut protein)	Starting dose chosen on pre-OIT threshold	**BP:** Dose doubled every 2 weeks until the end dose is reached.	Not specified	6 weeks	800 mg protein daily	Open food challeng	4/4(100%)	0/4(0%)	not tested

					MP: daily intake of maintenance dose				e			
Skripak et al. 2009	Milk (n=13)	72-204	Range: 1.14-34.3 mg CD: 71.4 mg (milk)	0.0114 mg (milk)	**MRP**: one day, dose doubled every 30 min until 50 mg. **BP:** dose doubled every 7 days **MP:** daily intake maintenance dose.	3-23 weeks	13 weeks	15 ml CM daily	DBPCFC	12/13(92%) *Control group: 0/7(0%) Placebo*	0/13(0%) *Control group: 0/7 (0%) Placebo*	not tested
Itoh et al. 2010	egg (n=6)	84-144	not specified	One tenth of pre-OIT threshold dose	**MRP:** 7-14 days, 3-5 times a day, 1.2-1.5 dose increase every 30 minutes. **MP:** maintenance dose twice a week	Not applicable	9-12 months	One whole egg	Open food challenge	6/6(100%)	0/6(0%)	not tested
Caminiti et al. 2009	Milk (n=10)	60-120	Range: 0.1-100 ml CD: 144.4 ml (milk)	0.5 ml (milk)	**BP:** dose doubled every week until the end dose is reached. **MP:** daily intake of maintenance dose	18 weeks	~4 months	200 ml CM daily	DBPCFC (3/10)	7/10 (70%) *Control group:* 0/3(0%) Placebo	1/10 (10%) *Control group:* 0/3(0%) Placebo	not tested
General	Milk (n=169) egg (n=90) cod (n=11) peanut (n=4)	7.2-660	Milk: 0.01-162.5 ml Egg: 5-700 mg Cod: 0.001-20g Peanut protein: 1-100 mg	Milk: 0.0006 ml – 5 ml Egg: 0.1 mg white powder – 1 g yolk Cod: 0.000033 mg Peanut: Starting dose chosen on pre-OIT threshold	**MRP:** 1-14 days, rapid dose increase **BP:** dose increase every 1-14 days till maintenance dose is reached **MP:** intake of the allergic antigen several times a week	Milk: 3 weeks – 12 months Egg: 3 months – 13 months Cod: 4-10 months Peanut: not specified	Milk: 13 weeks – 36 months Egg: 6-12 months Cod: not specified Peanut: 6 weeks	Milk: 15-200 ml Egg: one whole egg (60 g) Cod: 160 mg boiled Peanut: 800 mg protein	none, DBPCFC, SBPCFC or open food challenge	Milk: 106/155 (68%) (without Staden) Egg: 51/79 (65%) (without Staden) Cod: 8/11 (72.7%) Peanut: 4/4 (100%) Total: 181/274 (66%) Control group placebo/untreated: 43/155 (28%)	Milk: 27/155 (17%) (without Staden) Egg: 5/79 (6%) (without Staden) Cod: 2/11 (18.2%) Peanut: 0/4 (0%) Total: 34/274 (12%) Control group placebo/untreated: 0/155 (0%)	Milk: 0/1 (0%) (without Staden) Egg: 2/5 (40%) (without Staden) Cod: not tested Peanut: not tested Total: 11/31 (35%) Control group placebo/untreated: 7/20 (35%)

CD: cumulative dose, MRP: modified rush phase, BP: build-up phase, MP: maintenance phase, one drop is equal to 0.5 ml (drops are convent to milliliters), 0.05 ml ~ g contains 1.75 mg protein (mg protein is convent to mg whole milk and egg). The general final results do not contain the final results of Staden et al. because Staden et al. did not distinguish between milk and egg patients in their study. * SBPCFC in stead of DBPCFC

4.2. Discussion efficacy of OIT

In the past eight years several studies found promising results towards OIT (Table 1). Of the 274 patients, enrolled in the twelve clinical trials included in this review, 181 patients (66%) full desensitized during OIT treatment. When we take into count partial desensitization as a positive result as well, 215 patients (78%) benefit from OIT.

To examine if desensitization and/or tolerance are/is induced by OIT or by natural outgrow, five research groups included control groups in their study (Patriarca et al. 2003, Morisset et al. 2007, Staden et al. 2007, Skripak et al. 2009, and Caminiti et al. 2010). Immediately after OIT, OIT seems to have a significant positive effect on the allergy because more patients of the OIT group are remedied compared to the patients of the control group. Three studies (Rolinck et al. 2005, Buchanan et al. 2007, Staden et al. 2008) included an elimination diet after OIT in their protocol to examine if OIT has a transient of persistent effect. They did not find a significant persistent effect of OIT, since there was no difference in the number of patients who still were tolerant after a time interval and patients who were enrolled in the control groups. This indicates that OIT thus not induce tolerance and that OIT contribute towards desensitization.

However, there are some disadvantages. The results are supported only by a limited amount of studies and the general conclusions are debatable because there is much variation between studies (Table 1). First, all treatment protocols are performed in a different manner. Several studies started with a rush phase followed by a build-up phase, further followed by a maintenance phase (e.g.,Buchanan et al. 2007, Longo et al. 2007). Other studies used a protocol including only a build-up phase and maintenance phase (e.g.,Caminiti et al. 2010, Itoh et al. 2010). In addition, there is variation in up dosing schedules and duration of treatment. Some studies performed a more a more gradual increasing scheme of the allergic food compared to others. From subcutaneous immunotherapy it is know that the cumulative dose correlates with the outcome of tolerance development (Buchanan et al. 2007). If we would like to integrate OIT in clinic a more general protocol is needed. We have to find the finest protocol which we can induce the most positive outcomes.

Second, the mean age of children varies between studies. Several studies enrolled very young children (e.g.,Meglio et al. 2004, Morisset et al. 2007). In 2002, Host and colleagues showed that the recovery rate from cow's milk antigen was approximately 45 to 50% at one year, 60 to 75% at two year, 85 to 90% at three years and 90-95% at five and ten years. Therefore it is difficult to indicate if tolerance is induced by natural outgrow or by OIT in children younger than five year. All clinical trails should perform their study including merely children older than five. In addition, the number of spontaneous recoveries is poor (22% at two year) when cow's milk allergy is included in a multiple food allergy (Hill et al. 1999). None of the authors take this into account, however it is important to analyze because many allergic people have more than one allergy.

Third, none of the researches paid attention to dissimilarities between individual. For example, there are significant differences in the way men and women experience many diseases (Kim et al. 2010) and in the way an adult and child react on therapies. The effect of OIT on food allergy could be influenced by the characteristics of individuals. It is important to establish the effect of OIT for several groups of patients. For instance, when we know that OIT is more effective in children, we are able to decide desensitize children immediate after establishing the diagnose or we can decide not the desensitize elderly individuals with OIT.

Fourth, the severity of the food allergy differs among patients, because studies utilize dissimilar exclusion criteria. For example, Longo and colleagues (2008) enrolled only patients with a DBPCFC outcome lower than 8 ml for milk while Caminiti (2010) enrolled patient with a DBPCFC result lower than 100 ml for milk. Patients who are able to drink 100 ml milk do not have a severe allergy. It is likely that patients who already can tolerate 100 ml milk are more easily to desensitize and therefore it

is reasonable to assume that patients who are already able to drink 100 ml milk are more likely to become tolerant/desensitize. Furthermore, it is likely that the induction of tolerance/desensitization is settled more rapidly. Patients with a severe allergy, with a DBPCFC with a low outcome (+/- 10 ml), are the one who most likely need a treatment, because they react most heavenly when they accidentally ingest a small amount of the allergic antigen.

Finally, the definition for full desensitization varied between the different studies. Some studies hold a lower threshold compared with other studies (100 ml milk Rolinck versus 200 ml milk Caminiti). By this discrepancy some researchers could achieve positive results much earlier. In addition, it is possible that children who are able to drink 100 ml milk experience symptoms when they ingest a higher amount of the allergen. Therefore children who are able to ingest 200 ml are probably better protected.

Researchers should perform their studies without these discrepancies, so results of various studies could be better compared. They should arrange a clear definition for food allergy, a DBPCFC threshold to enroll patients in their studies, and they should determine a value for when an allergy is resolve and when the person is desensitize and/or tolerant.

To be able to apply this treatment in clinic, it is important to obtain more information by further research. The previous described trials in some cases fall short of not performing DBPCFC to establishing the threshold dose, not including control groups, not including a time interval with elimination diet after OIT, and/or not including a reliable number of patients. In addition, the mean age of the volunteers is an issue. Control groups are needed to establish the actual effect of OIT, because examine if desensitization/tolerance is induced by OIT or natural outgrow. A time interval (elimination diet) after OIT is important to differentiate between desensitization and tolerance and a large group of volunteers is preferred to obtain significant and well supported results.

Another shortcoming of the presented studies is that they took a long time to proceed, because it is laborious to follow patients in time when the protocol is time consuming. Motivation from physicians is needed to perform an excellent clinical trail.

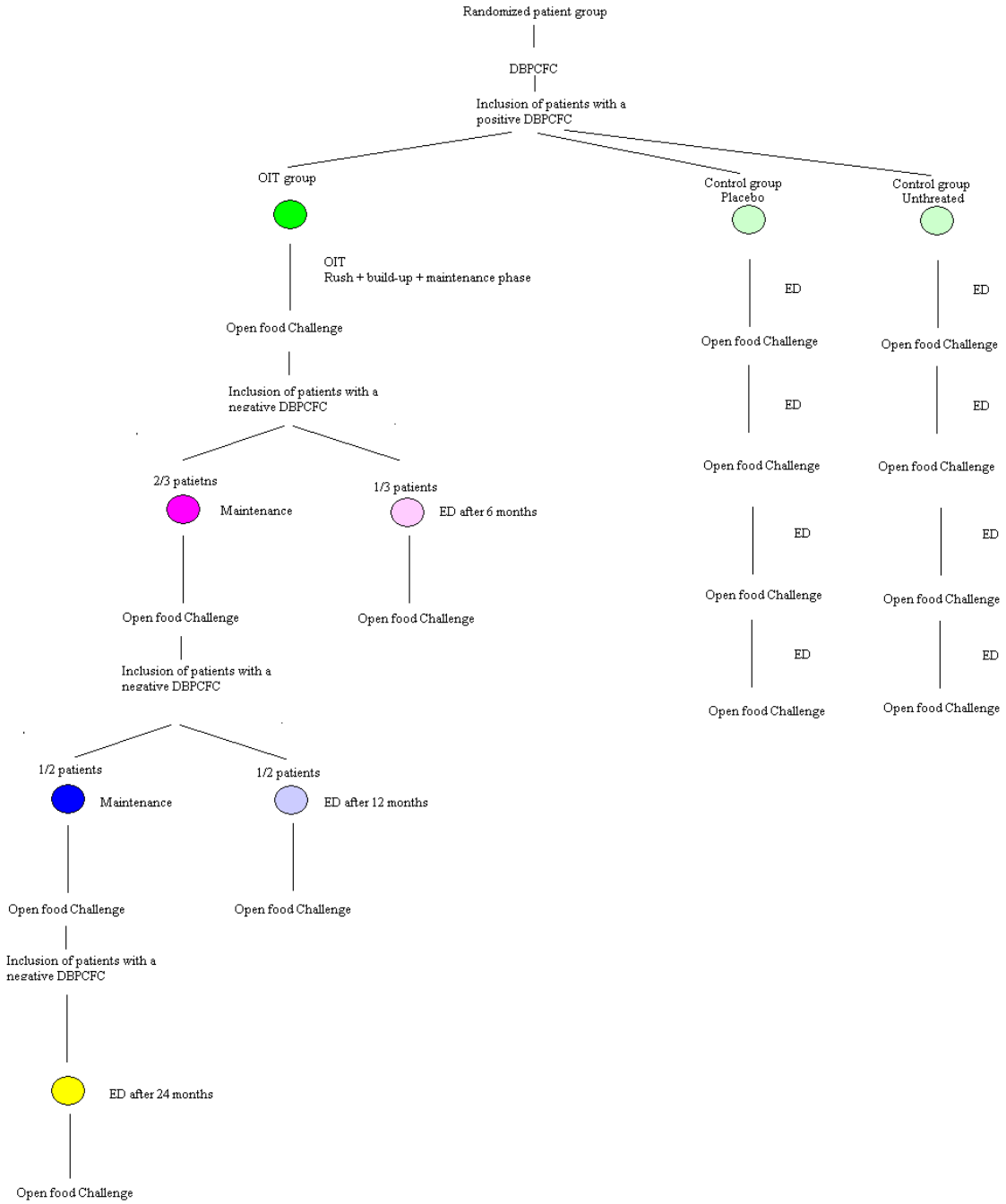
Long lasting protocols are in as well not pleasant for patients, because it put a lot on patients. How it is possible that patients not discontinued the intake of food like egg, fish or peanut? Patients must be motivated to participate in a trial. Fortunate, most phases of OIT can be performed at home. In addition, a rush phase is introduced by several groups to reduce the time of the protocol (Aragones et al. 2007, Buchanan et al, 2007, Longo et al. 2008, Skripak et al. 2009, Itoh et al. 2010). With a rush phase patients are desensitized in several weeks. Results of studies who performed a rush phase are comparable with long lasting protocols (e.g., Skripak: 92% with rush phase versus Morisset: 88.9% without a rush phase).

Up to now no difference is found in efficacy between various allergic foods. However, in most studies OIT has been performed in milk and egg allergic children. Only a few papers have been written about OIT for peanut allergy. In this review only the article of Clark could be used because it was the only article about peanut allergy who met the inclusion criteria. Therefore, especially more research to OIT for peanut allergy is needed. This is also important because natural tolerance development is less frequent in this allergy and the majority of children remain allergic until adulthood (Beyer et al. 2008, Spergel et al. 2000).

To summarize what type of research is needed in the future, I made a proposal for the so called ‘perfect trial’ (Figure 1). In this trial only children with a positive DBPCFC will be enrolled in the study. These children will be divided among three groups (OIT group, unthreaded control group, placebo control group). The children in the OIT group will undergo a rush phase, a build-up phase and a short maintenance phase. We assume a rush phase is preferred because it will accelerate the treatment and will increases safety and the dose might be increased more rapidly.

One third of the OIT group will undergo directly, after this phase, an elimination diet(ED) to establish the effect of a short cure on tolerance. It is expected that desensitization disappears after this ED, because this is seen in clinical trails examining the effect immunotherapy for other allergies then food allergies. Just a rush phase in combination with a build-up phase is probably too short to induce tolerance and raises only the dose threshold. In case of venom allergies the maintenance phase is more than three years (Diwaka et al. 2008, van Halteren et al. 1998). In two third of the patients of the OIT group, the build-up phase is followed by a maintenance phase. Half of the patients of this group will have to take food for a longer period before an elimination diet will be started to establish the effect of regular intake on the threshold and the effect on long-term tolerance.
During and after the elimination diets an open food challenge will be performed to establish if desensitization is induced, persistent or transient. For instance, this challenge will be utilized every two weeks during and after the ED. When it seems the effect of OIT is persistent we could enlarge the intervals between the tests. An open food challenge is preferred, because a DBPCFC takes a lot of effort and the open test is reliable because the allergy of the patients is establish before OIT.
In the perfect trial two kinds of control groups are included to evaluate whether desensitization/tolerance has been is induced by OIT or natural outgrow. With a patient group, receiving a placebo, the placebo effect of the therapy will be evaluated. Children of 5 years and older will be enrolled in this study to reduce the chance of natural outgrow.
To examine if desensitization/tolerance might be faster induced by OIT than natural outgrow a randomized patient group younger than five years is preferred. It is implicated that OIT will accelerate the process of natural outgrow. To study this fact the same protocol could be followed with this special group.

Figure 1: The perfect clinical trial



The perfect clinical trail. DBPCFC: double blind placebo controlled food challenge, OIT: oral immunotherapy, Rush: rapid dose increase, ED: elimination diet.

4.3 The safety of OIT

Patriarca and colleagues enrolled 59 patients in their clinical trial and performed 66 treatments. Of these patients 51.1% of the patients experienced side-effects, such as urticaria, angioedema and/or abdominal pain. These side-effects were kept under control with medication. Sodium cromolyn was given 20 minutes before food ingestion if clinical symptoms, such as pruritus or erythema, were observed. There was no need for adrenaline administration or hospitalization. 16.7% of the patients had to stop with the therapy because of uncontrolled side effects.

Meglio and colleagues (2004, 2008) tried to desensitize 21 children with cow's milk allergy. After a long lasting protocol fifteen children were desensitized whereby eight children without any symptom. The other patients had symptoms like abdominal pain, pruritus, erythema and asthma by quite a few different doses of CM. Three children could not follow the desensitization protocol because they presented severe side-effect. Three other children could not finish the protocol, nevertheless they reached partial tolerance. During treatment all patients administered certizine to control symptoms. Three weeks after the end of the protocol, the intake of certizine was discontinued. None of the children presented any symptoms after stopping certizine intake while they continued the intake of CM. After finishing the protocol, patients were monitored during a period of more than four years. During this period none of the patients needed adrenaline or required emergency care. Some children suffered from mild symptoms, but these symptoms were resolved by antihistamine.

Rolinck and colleagues (2005) included three patients in their investment. These patients and their parents were trained in medical treatment and equipped with drugs. There was also a telephone number available to contact the coordinator round the clock. This safety procedure is applied in many other studies where patients follow an up dosing protocol at home (e.g., Clark et al. 2008). In this investment patients underwent a build-up phase and a maintenance phase. By minor symptoms at some stage in the build-up phase, the patients had to extend this phase. After the maintenance phase two patients follow an elimination diet of two months and experienced symptoms like urticaria and rhinoconjunctivis during a DPBCFC subsequently. At the end of their study Rolinck and colleagues result that their cases indicate that for safety reasons a daily intake should be recommended.

Morisset (2007) tried to desensitize children with milk or egg allergies. Of the children with milk allergy three children discontinued their diet due to clinical reactions. Two of them had a positive oral challenge and one was not re-challenged. Children with egg allergies also suffered from the therapy especially in the first weeks. Cooked egg was used in this study because it is better tolerated in egg allergic subjects.

In the study of Aragones (2007) four patients with CM allergy were included. The patients enrolled in this study were particular young (nineteen months, two year, two year, and five year). During the protocol all patients presented symptoms like erythema or urticaria elements. Most symptoms were solved in a few minutes without the use of medication. One patient presented cough and wheezing with one dose and this symptom was controlled by salbutamol. Another patient interrupted the protocol since he presented abdominal pain and oropharyngeal itching. After eleven days he started again with a slow increase of the doses each three to seven days.

Staden and colleagues (2007) included 45 patients in their study and divided them in an OIT group and a control group. During the study, all children in the OIT group had side effects. Twenty-one patients of this group had mild symptoms, like wheals, tingling sensation, nausea and eczema, easily controlled with oral antihistamines. Four patients showed moderate symptoms, like urticaria, bronchial obstruction, angioedema, inhibited with antihistamine and steroids. None of the patients in the OIT group suffered from severe reactions in contrast to the control group. In the control group, one person had a severe reaction after accidental ingestion of the allergic food. Staden et al. provide a reason why side-effects are common in their study: 'We did not used antihistamines on regular basis to mask side-

effects'. Furthermore, many patients suffer from 'augmentation factors' like infection, exercise and pollen allergy. Staden et al. indicate that this is important to take this in account. In addition they indicate that careful monitoring during OIT is mandatory.
Seven subjects, without a history of anaphylaxis to egg, were enrolled in the study of Buchanan (2007). During the modified rush phase symptoms were generally mild, however five patients administered antihistamine and one subject received intravenous fluids. Just one patient did not demand any treatment in the period of this phase. During the build-up phase subjects tolerated all daily doses without significant difficulty. None of the patients experienced symptoms in the maintenance phase for the period of the two year. At the end of the study Buchanan et al. conclude that their study provide evidence that OIT can only be safely used for patients with egg allergy without a history of anaphylaxis to egg up to now.

Longo and colleagues (2008) included only children in their study with outstandingly severe reactions after ingesting trace amounts of cow's milk proteins. Because these patients are high at risk for severe side-effects during OIT, children were excluded if their parents had a history of unreliable management of complications and if they had limited availability to emergency facilities in the area. In addition, children with poorly controlled asthma were excluded. All children were administered with anti-histamine daily. When patients had reached the single dose of 150 ml the ingestion of antihistamine on regular base was reduced over four weeks. Despite these strict rules and measures almost all patients had allergic reaction during treatment. During hospitalization (rush phase) intramuscular epinephrine was administered four times with four children and nebulized epinephrine was administered by eighteen children to control side-effects. During home dosing two children needed hospitalization because of severe side-effects. In finalizing the study a large part of the treatment group was sent home with a lower maintenance dose because of frequent allergic reactions. Longo concluded that the data of this study is too limited to estimate true risk or near-fatal events.

Clark and colleagues (2008) performed clinical trial, which includes four children with peanut allergy. During OIT no severe reactions were reported and adrenaline was not required. When a patient experienced a mild reaction this subject was advised to continue taking the current dose. In two occasions, subjects experienced mild abdominal pain while receiving a previously tolerated dose. However, this was associated with the subject being tired or performing enthusiastic exercise within 1 h of taking the dose. At the end of the study all children reached the maintenance dose.

During the study of Skripak (2009) 2437 active OIT doses and 1193 placebo doses were given. These doses have respectively caused 1107 (45,4%) versus 134 (11.2%) total reactions. Multiple system reactions were rare (1% of the doses) in the OIT group and there were no multiple system reactions in the placebo group. The frequency of symptoms and medicine administration varied widely among patients. For example, one patient received one third of all doses diphenhydramine. Although reactions after ingestion of the allergic food were common and all active treated patients experienced at least one adverse event, nearly 90% of all acute reactions were temporary and required no treatment. According to Skripak and colleagues this amount of side-effects is acceptable, but further study is essential if we want to utilize this method in clinic.

The group of Itoh (2010) performed an OIT trial including six patients allergic to egg. All patients enrolled in this trial had atopic dermatitis and asthma. During DBPCFC all children experience side-effects and all children experience side effect during rush OIT. During rush phase most symptoms were observed regardless of changes to scrambled egg. Side-effects were controlled by antihistamine if needed. Only one patient required oral steroid because she experienced severe urticaria all over her body. The reason why these children suffer from side-effects is according to Itoh, due the fact that these patients had severe food allergy and did not receive oral antihistamine on a regular basis so allergic reactions were not masked. However, rush SOTI doses were increased in hospital, so patients had no need to bother about allergic reactions at home. Itoh conclude that rush OIT is a safe and effective way to desensitized patients from severe food allergy to egg.

Caminiti and colleagues (2010) applied oral immunotherapy on ten patients. During this treatment almost all patients experienced side-effects. Eight out of ten patients suffered from symptoms at various stages in CM desensitization and four of them needed medication (adrenaline, steroids, antihistamine, and salbutamol). Before OIT was started, a DBPCFC was performed. Two patients could not undergo this challenge due to severe reactions. One of these patients failed the therapeutic approach and the other one reach partial tolerance. In this study side-effects occur independently of age. Caminiti and colleagues concluded that the procedure is not devoid of severe adverse events. However the risk of having a reaction due to inadvertent ingestion is certainly higher than the risk of a reaction during a medically supervised desensitization.

Table 1: Overview of safety data from oral immunotherapy trials: the table summarized the characteristics of all included papers in terms of safety.

Author, year	Age in months	Number of patients started OIT	Patients who stopped therapy by uncontrolled side-effects	Severity of symptoms	Antihistamine constant given during OIT	Medication used to resolve side-effects	Need for hospitalization
Patriarca et al. 2003	36-660	Milk: 29 Egg: 15 Cod: 11	Milk: 5 Egg: 2 Cod: 1	Grade 1/2: 28/55 (51.1%) Grade 2 (not controlled with medication): 8/55 (14.5%)	No	-H1-antihistamine -Sodium cromolyn	No
Meglio et al. 2004	63-122	21	3/21 (14%)	No symptoms: 8/21 (38%) Local: 0/21 (0) Grade 1: 5/21 (24%) Grade 2: 5/21 (24%) Grade 3: 3/21 (24%) Grade 4: 0/21 (0%)	Yes	Antihistamine	No
Meglio et al. 2008 (follow up of Meglio et al. 2004)	6-3-122 (4 yr and 8 months later)	16 of the 21 have continued the ingestion of milk during follow up	Not applicable	No symptoms: 12/16 (75%) Local: 0/16 Grade 1: 1/16 (6.25%) Grade 2: 3/16 (18.75%)	Not applicable	Antihistamine	No
Rolinck et al. 2005	58.8-154.8	3 CM=1 HE=2	0/3 (0%)	No symptoms: 0/3 (0%) Local: 0/3 (0%) Grade 1: 1/3 (33%) Grade 2: 2/3 (66%) Grade 3: 0/3 (0%) Grade 4: 0/21 (0%)	No	Not specified	No
Aragones et al. 2007	19-60	4	0/4 (0%)	No symptoms: 0/4 (0%) Local: 0/4 (0%) Grade 1: 2/4 (50%) Grade 2: 2/4 (50%) Grade 3: 0/4 (0%) Grade 4: 0/4 (0%)	No	-Salbutamol	No
Staden et al. 2007	7.2-154.8	25 CM=14 HE =11	0/25 (0%)	No symptoms: 0/25 (0%) Local symptoms: 0/25 (0%) Grade 1/2: 21/25 (84%) Grade 3: 4/25 (16%)	No	-Oral antihistamine -Steroids	No
Buchanan et al. 2007	14-84	7	0/7 (0)%)	No symptoms: 0/7 (0%) Local: 0/7 (0%) Grade 1: 1/7 (14%) Grade 2: 5/7 (71%) Grade 3: 0/7 (0%) Grade 4: 1/7 (14%)	No	-Antihistamine -Intravenous fluids -Oral diphenhydramine	No

Longo et al. 2008	60-204	30	3/30 (10%)	*No symptoms: 0% Local: 62% Grade 1: 13% Grade 2: 16% Grade 3: 9% Grade 4: 0%	Yes	-Intramuscular epinephrine -Nebulized epinephrine -Oral steroids -Antihistamine	Yes (2/30)
Skripak et al. 2008	72-204	13	1/13 (8%)	**No symptoms: 54.4% Local: 35.7% Grade 1: 0.9 % Grade 2: 18.7% Grade 3: 8.1% Grade 4: 1.2%	No	-Diphenhydramine -Oral antibiotic -Epinephrine	No
Clark et al. 2009	108-156	4	0/4 (0%)	No symptoms: 1/4 (25%) Local: 0/4 (0%) Grade 1: 0/4 (0%) Grade 2: 3/4 (75%) Grade 3: 0/4 (0%) Grade 4: 0/4 (0%)	No	Not specified	No
Itoh et al. 2010	84-144	6	0/6 (0%)	No symptoms: 0/6 (0%) Local: 0/6 (0%) Grade 1: 0/6 (0%) Grade 2: 3/6 (50%) Grade 3: 3/6 (50%) Grade 4: 0/6 (0%)	No	-Antihistamine -Oral steroid	No
Caminiti et al. 2009	60-120	10	2/10 (20%)	No symptoms: 2/10 (20%) Local: 0/10 (0%) Grade 1: 2/10 (20%) Grade 2: 4/10 (40%) Grade 3: 1/10 (10%) Grade 4: 1/10 (10%)	No	-Adrenaline -Antihistamine -Corticosteroids -Salbutamol	Not specified

CM: cow milk, HE: hen's egg. For each patient the most severe reaction is counted and used in the table. * Total of 706 reactions, percentages derivative of total reaction, most severe reaction each dose.
**Total of 2437 reactions, percentages derivative of total reactions, sometimes more reactions each dose.

4.4. Discussion the safety of OIT

It is difficult to draw conclusions about the results of OIT related to safety because different studies established the earnest of allergic reactions in various ways. The main problem is subjectivity. Different authors have a different definition for suffering and different patients have different thresholds for pain. In addition, older children are more diligent in reporting symptoms. Notwithstanding this difficulty, there is one major general fact: almost all children enrolled in the studies suffer from side-effects and these side effects are seen at all dose levels and in all phases (rush, build-up and maintenance). To compare the different articles we linked the symptoms to a certain grade severity.

Although the frequency of side-effects is very high in all studies it is seen that patients in some groups (e.g., Meglio et al. 2004, Skripak et al. 2008) less affected compared to other studies (e.g., Itoh et al. 2010). How this discrepancies arise is not fully understood, nevertheless there have been devised a few options. First, patients could have more than one allergy and side-effects could be induced by the other allergies (Hill et al. 1999). Second, daily intake of the allergic protein could improve safety (Rolinck et al. 2005). Third, several studies (e.g., Aragones et al. 2007, Buchanen et al. 2007) performed a rush phase (daily intake, several doses each day) to increase the threshold of the patients. They declare that a higher threshold protects the patients to severe side-effects. Fourth, in some investments (e.g., Meglio et al. 2004, Skripak et al. 2008) medication is given on regular base to suppress symptoms. In the study of Skripak et al. all participants took daily long-acting antihistamine and in the study of Meglio all patients used certizine. In the study of Meglio et al. eight of the twenty-one patients showed no side-effects and this is high compared to other studies. Despite the regular intake of antihistamine all patients of Skripak had to deal with side-effects. Nevertheless, Skripak enrolled only patients with a severe allergy. Finally, augmentation factors such as exercise and illness could induce side-effects (Staden et al. 2008). Despite the high prevalence of side effect just a few children could not finish OIT and just a few patients suffered from severe side effect and/or needed hospitalization.

Due the large amount of side-effects it could be safer to perform OIT in hospital. Caminiti declares that OIT in hospital is safer and Itoh indicates that patients had no need to bother about allergic reactions at home because doses were increased in hospital. Nevertheless, performing OIT at home is more patient-friendly.

Some papers (e.g., Rolinck et al. 2005, Staden et al. 2007, Longo et al. 2008, Clark et al. 2008) reviewed in this paper describe the importance of safety measures and the presence of a motivated colleagues. Patients and parents must be trained in medical treatment of allergic reactions and equipped with drugs. In almost all studies there was a telephone number available to contact the coordinator at all times. This is especially critical for patients and parents who followed an OIT schedule at home. When patients or their parents think about contribute an OIT trial, they must be alert of the risk.

Overall, it is difficult to say if this therapy is safe enough to apply in clinic. First of all more research is needed. To collect comparable information about the safety of this therapy I think it is advisable to use a reliable method to establish the severity of side-effects. Furthermore, it is important to examine the effect of OIT on different groups with discrepancies in age, kind of allergy, multiple allergies etc. Further, it is important to have a good medical team to perform the trail. In addition, motivated patients (and parents) are important, because the protocol requires a lot of them. Probably, it is advisable to perform the trails in hospital for safety and it is maybe recommendable that patients get medication constantly during there therapy to suppress side-effects. It is likely that more patients can complete the therapy, when they lead less.

5. The mechanism of desensitization and/or tolerance induction

In this chapter I will describe the mechanism of oral tolerance, food allergy and the mechanism of desensitization and/or tolerance after oral immunotherapy. First I will briefly describe how it is possible that despite the extent of protein exposure, very few patients suffer from food allergies. Second I will describe how an allergy is induced. Finally I will illustrate the mechanism of how desensitization and/or tolerance will occur using the twelve studies included in this review.

5.1 Oral tolerance

The gastrointestinal tract is the biggest immunologic organ of the human body. The surface epithelium of this tract (the lumen) is exposed daily to multiple dietary proteins and bacteria. In spite of these exposures, only a small percentage of individuals have food allergy. This small percentage is due to the development of oral tolerance to dietary proteins. Antigen exposure in the gut leads to a protective local and systemic immunologic responses which encourage immulogic tolerance (Chahade et al. 2005, Burks et al. 2008, Vickery et al. 2009).

Following oral ingestion, first dietary proteins undergo modification by digestion in the lumen which often results in the destruction of immunogenic epitopes. Interruption of this process disturbs tolerance and might lead to hypersensivity (Burks et al. 2008, Vickery et al. 2009). Micheal et al. (1989) examined the influence of digestive enzymes on the tolerogenic properties of an orally administered protein antigen in a mouse model. The protein was tolerogenic when administered orally but immunogenic following ileul administration, administration in the final section of the small intestine. Orally administered proteins were degraded by the digestive system while the degradation in the ileum was limited. They conclude that to acquire tolerogenic properties, an orally administered protein must be fist degraded by proteolytic enzymes in the lumen. Immunogenic proteins, who escape luminal digestion, can interact with the mucosal immune system and lead to an allergy (Burks et al. 2008, Vickery et al. 2009). This can occur in at least three ways:

(1) Antigens can be sampled by lamina propria dendritic cells (DCs) into the lumen (Fig2a).
(2) Antigens might be taken up by Microfold cells (M cells), located in the epithelium, and delivered to lamina propria DCs (Fig2b).
(3) Epithelial cells, expressing MHC class II molecules, may take up dietary protein antigens and act as a nonprofessional antigen-presenting cell (APCs). In addition, soluble antigens might cross through paracellular channels in damaged gut epithelium to encounter T cells in the lymphoid tissue (Fig2c).

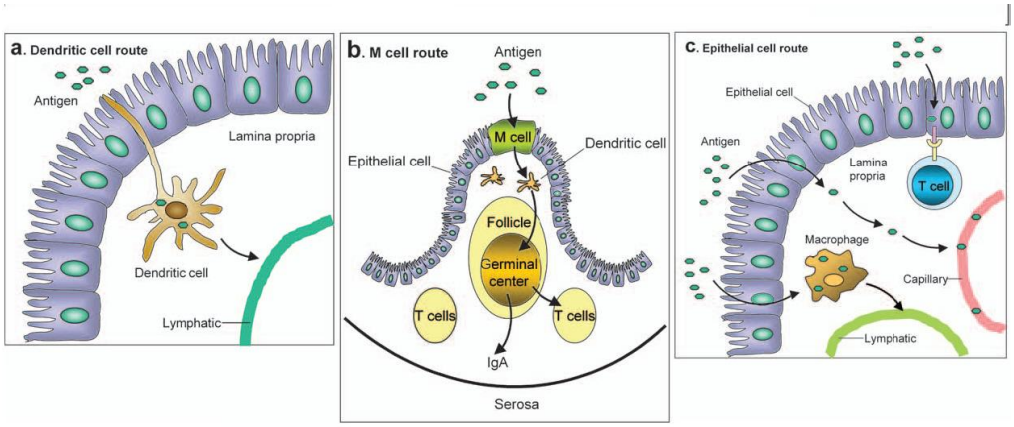


Figure 2 (Chahade et al. 2005): A. Antigens sampling by lamina propria dendritic cells (DCs) into the lumen. B. Antigens uptake by Microfold cells (M cells) and delivering to lamina propria DCs. C. Epithelial cells, expressing MHC class II molecules, may take up dietary proteins antigens and act as nonprofessional antigen-presenting cells (APCs). Furthermore, soluble antigens might cross through paracellular channels in damage gut epithelium to encounter T cells in the lymphoid tissue.

Subsequently, activated dendritic cells enclose a central role in the induction of tolerance (Burks et al. 2008, Vickery et al. 2009). Tolerogenic local factors influence the dendritic cell-T cell interaction which results mostly in suppression of the immune response. Depending on the characteristic and concentration of the antigen, suppression is induced in different ways. A low dose of allergen persuades tolerance driven by regulatory cells (Th3 cells, Tr1 cells, CD4+DC25+ cells, CD8+ cells and natural killer cells), while a high dose of allergen favors anergy-driven tolerance or clonal deletion. However, low-dose and high-dose tolerance could have overlapping functionality.

Regulatory T cells can suppress immune responses by the excretion of suppressive cytokines like IL-10, IL-4 and TGF-β. Due to these signals they inhibit effector cells like cytotoxic T cells, T-helper cells (Th-cells), and B cells (Fig3a). For instance duodenal mucosal lymphocytes from children with milk induced gastrointestinal diseases were cultured in vitro in the presence of milk. Upon restimulation with milk protein, these lymphocytes released the Th2-asociated cytokines IL-5 and IL-13 and very low amounts of TGF-b and IL-10, suggesting a defect in inducible regulatory T cells function (Beyer et al. 2002). More evidence of the role of regulatory T cells is found by Karlsson et al. (2004) and Ozcan et al. (2008). Ozcan et al. found that patients with regulatory T cells derived from the thymus and expressing a mutated form of the transcription factor forkhead box P3 (FoxP3) develop the immunodysregulation polyendocrinopahty enteropathy, X-linked (IPEX) syndrome. Patients with this syndrome have very high Th2 responses, high IgE levels and allergies. Karlsson et al. reported that children who had outgrown cow's milk allergy had higher numbers of T regulatory cells than children who had remained allergic.

Anergy, a lack of reaction by the body's defense mechanism to foreign substances, can arise through T-cell receptor ligation in the absence of costimulatory signals provided by soluble cytokines, such as IL-12 or by the interaction between receptors on T cells (CD28) and counter-receptors on APCs (CD80 and CD86) (Fig3b). During a 'normal' immune response, for example against pathogenic bacterium, ligation of the T-cell receptor with peptide-MHC complexes in the presence of appropriate costimulatory molecules (CD80 and CD86) and cytokines (IL-12) induces the activation of effector cells. Deletion, induced by a high dose of antigen, occurs by means of FAS-mediated apoptosis, which can be blocked by the proinflammatory cytokine IL-12 (Fig not shown).

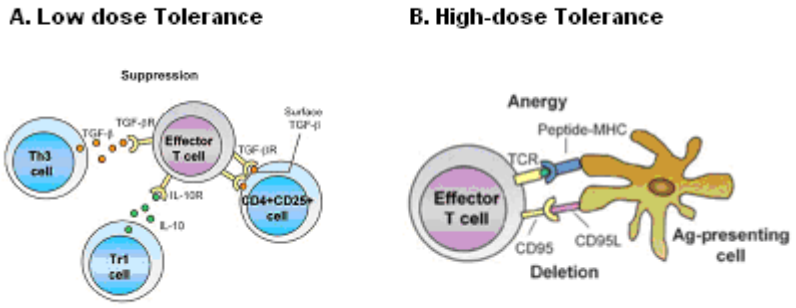


Figure 3 (Chahade et al. 2005): A. Suppression of effector cell by the excretion of suppressive cytokines by regulatory T-cells. B. Anergy induction by ligation in of T-cell receptor in the absence of costimulatory signals.

Up to now not all facts about the role of antigen presenting cells in the induction of tolerance is comprehensible. Recently, new factors in the environment of dendritic cells are found which participate in the process of tolerance induction. These factors have effect on which dendritic cells become activated, how dendritic cells participate in tolerance induction and the way of response of effector cells (anergy, clonal deletion, effector cell activation. For instance there is evidence that CD11C+ mucosal dendritic cells change over from naïve T cells to regulatory T cells by the vitamin A metabolite retinoic acid in the presence of TGF-β (Scott et al. 1980).

When tolerance to a given dietary antigen is not established or is broken down, food hypersensitivity is induced. Several factors might induce a breakthrough of tolerance: antigen properties, route of exposure, age of the host, genetics etc. These factors can lead to disrupting of the digestion process and protection from both acid and enzymatic digestion of the antigen. Furthermore, it can induce defects in the regulatory T-cell activity.

5.2 The mechanism of food allergy

Oral tolerance to dietary proteins is crucial to prevent the development of food hypersensitivities. Disruption of the balance between harmful pathogens and recognition of harmless food proteins might induce food allergies. The induction of food allergy is called sensitization.

In case of sensitization an allergen, for example B-casein and K-casein (milk proteins), is absorbed by the gastrointestinal tract and presented by the APC to a TH2 cell for the first time, because the induction of tolerance has failed. The central role of Th2 in the development of food allergies has been established, signifying a Th2 bias in predisposed individuals (Knippels et al. 2004). Furthermore, in mice, genetic susceptibility to food allergy has recently been linked to differential Th2-Th1 responses (Morafo et al. 2003). The Th2 cell will be activated and the activated Th2 cell activates B cells by the excretion of IL-4 and IL-13. The activated B-cell secretes specific IgE and then IgE molecules attach mast cells. Now, sensitization is induced.

The food allergy is reflected by a provocation. During a provocation allergens are absorbed by the gastrointestinal tract and subsequently attach to the IgE molecules, which are bound to mast cells. By this binding the mast cell will be activated and will secrete mediators inducing an acute allergic reaction, with eg., wheezing, bronchoconstriction and cardiovascular collapse. Th2 cells are activated as well. These Th2 cells induce chronic diseases, eosinophil activation and airway hyperreactivity.

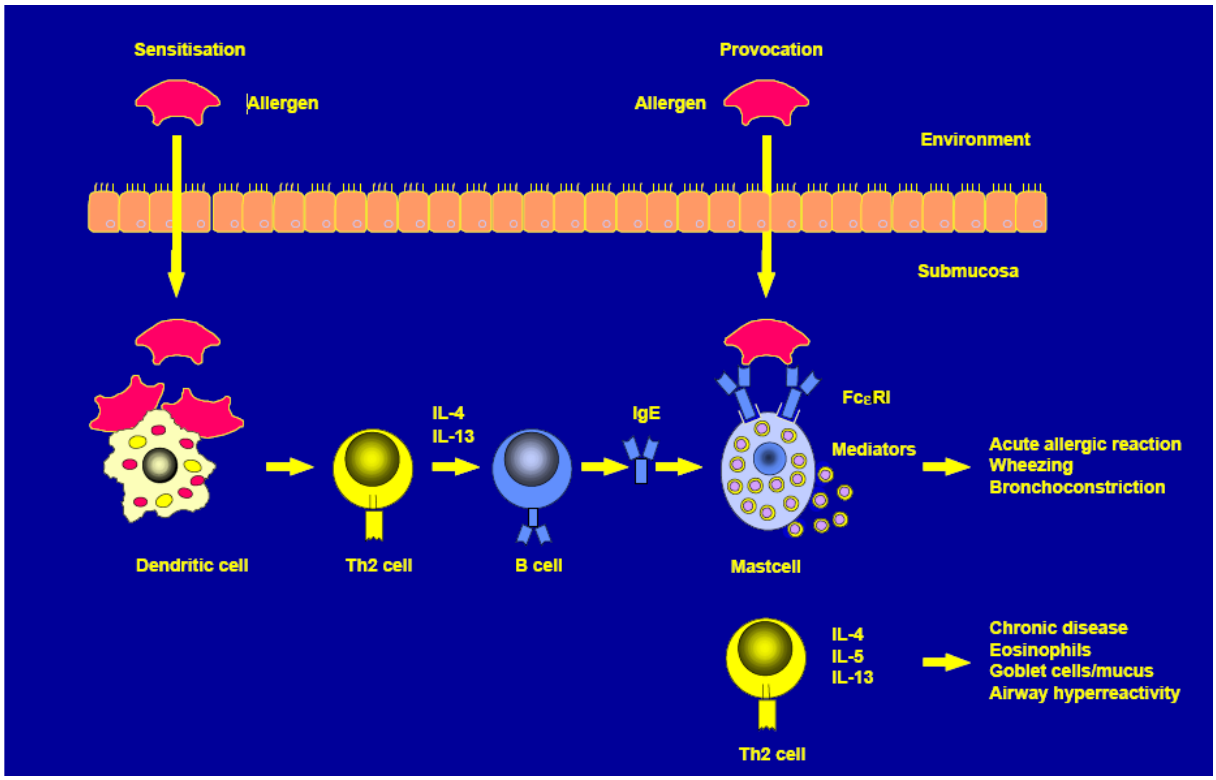


Figure 4 : The mechanism of the induction of an allergy: sensitization and provocation (The figure comes from a lecture of A van Oosterhout given in 2009, immunolgy II).

5.3 The mechanism of oral desensitization or tolerance induction by OIT

Up to now it is uncertain if OIT induces only threshold rising or is also able to induce tolerance. Certainly it is unclear which mechanism holds behind threshold rising and/or the induction of tolerance. Only a small number of studies included in this review give attention to the underlying mechanism of oral desensitization and/or tolerance induction by OIT in human.

In the study of Patriarca et al. (2003), Aragones et al. (2007), Staden et al. (2007) and Itoh et al. (2010) specific IgE (sIgE) decreased significantly. Staden et al. find that sIgE decreased significantly over time in children who developed natural tolerance and who reach (partial) tolerance by OIT. They did not find a significant change in sIgE in children who had to stop OIT because of side-effects. Meglio and colleagues (Meglio et al. 2004, Meglio et al. 2008) did not found significant changes in IgE between the beginning, after three months and at the end of the protocol. After a follow up of more than four year specific IgE was decreased significantly. In addition, Skripak found no significant change within any group in CM-specific IgE antibodies. He discussed that it is possible that the duration of treatment in their study was not long enough to see a decrease in serum IgE antibody levels. This supported the outcome of Meglio where IgE is finally decreased after four year. Buchanan, Rolinck and Longo found a reduction of IgE levels; however they did not find a significant reduction. From venom allergies it is know that the concentration of IgE fluctuate during the seasons and several years. These fluctuations were also seen in the studies included in this review. In addition, by clinical trails with immunotherapy for other allergies (eg, venom, pollen) there is seen a decrease in IgE after OIT (Brasch et al. 2009, Cantani et al. 2005).

Patriarca et al. (2003), Buchanan et al. (2008), Skripak et al. (2009) and Itoh et al. (2010) also measured specific IgG levels. In the study of Patriarca, specific IgG4 levels increased significant after eighteen months. In the study of Buchanan egg-specific IgG levels decreased from baseline increased significantly from baseline to 24 months. In the study of Skripak and colleagues there was a median increase from baseline in the active group. An increase in specific IgG4 (and IL-10) levels only in the active group, suggesting the possibility of immune regulation through regulatory T cells according to Allam et al (2003) and Enrique et al (2008). Furthermore, increased levels of specific IgG4 with or without decreased IgE have been associated with successful venom immunotherapy (Clements et al. 1998). The role of IgG in the induction of immunotherapy is not clear yet.

The hypothesis, currently accepted, is that oral tolerance is first achieved through antigen-specific mast cell desensitization, followed by a Th-mediated process with normalization of IgE levels. Decrease IgE levels, founded in the clinical trails included, support this hypothesis. The role of Th-cells is supported by Jones et al. (2009). Jones et al. examined the effect of OIT on patients with peanut allergy and investigated the PoxP3+ T cells population by flow cytometrie. During OIT, the number of FoxP3 T cells increased approximately 1.5- fold in peanut stimulated cells at six and twelve months and decrease thereafter to baseline by twenty months. This change did not reflect the transition toward a Th-cell profile. However, according to Jones et al. the early induction of these cells and the associated increase in IL-10 indicate an immulogic change induced by OIT, with transition away from a Th2-type profile. Furthermore, mice who are genetic susceptibility to food allergy has been linked to differential Th2-Th1 responses by Morafo et al. (2003) and Knippels et al. (2004) found a Th2 bias in predisposed individuals for allergies (also describe in chapter 5.1).

It is essential to clarify the mechanism since it may help increase the efficacy rates of OIT

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