# Immunotherapy-possibilities and dangers in food allergy

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## Abstract

Food allergy is a growing health concern in the westernized world with approximately 6% of children suffering from it. A lack of approved treatment has led to strict avoidance of the culprit food proteins being the only standard of care. Nowadays in-depth research is conducted to evaluate the possible use of allergen-specific immunotherapy (SIT) as an active therapeutic option for food allergy. Various routes of administration for the immunotherapy are investigated, including subcutaneous, oral, sublingual, and epicutaneous and some appear to be successful in inducing desensitization. Most research has been conducted with oral immunotherapy due to its efficacious and relatively safe profile. Increasing interest is dedicated to safer and more convenient approaches, such as sublingual and epicutaneous SIT, however, doubts exist about their possible capacity to induce tolerance. Although the underlying mechanisms of successful desensitization with SIT are not yet clear, evidence suggests that those involve suppression of mast cell and basophil reactivity, reduction in allergen-specific IgE and increase in allergen-specific IgG4 antibodies, as well as stimulation of natural or *de novo* induced regulatory T cells and skewing of the T cell response from the pro-allergic $T_h2$ to the $T_h1$ phenotype. The high frequency of allergic adverse reactions of the various approaches and the inability to achieve permanent oral tolerance have highlighted the need of refinements in the strategies and the potential use of tolerazing adjuvants. A promising strategy for preventing IgE cross-linking and thus enhancing safety of SIT, while still activating T cells, is the use of tolerogenic peptides. The implementation of a tolerizing $T_{reg}/T_h1$-polarizing adjuvant, such as a specific mixture of non-digestible oligosaccharides, into immunotherapy approaches has the potential of not only increasing the chance of achieving permanent state of tolerance, but also improving the safety and tolerability of the therapy. Immunotherapy for food allergy is still not ready for the clinic, but current and upcoming studies are dedicated at collecting enough evidence for the possible implementation of allergen-specific immunotherapy as a standard treatment for food allergy.

***Key words: immunotherapy, food allergy, tolerogenic peptides, non-digestible oligosaccharides***

***List of abbreviations:*** gastrointestinal tract, GI tract; peripheral blood mononuclear cells, PBMC; allergen-specific immunotherapy, SIT; subcutaneous immunotherapy, SCIT; oral immunotherapy, OIT; sublingual immunotherapy, SLIT; epicutaneous immunotherapy, EPIT; Payer's patches, PP; mesenteric lymph nodes, MLN; immunoglobulin free light chains, IgLC; short-chain galacto- and long-chain fructo-oligosaccharides, scGOS/lcFOS

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## Introduction

Food allergy is a growing health concern in the western world. Statistics have determined that approx. 6% of children, compared to 3-4% of adults, suffer from allergy to food proteins (1). In reports from the Centres of Disease Control and Prevention in the USA, the prevalence of food allergy in children is determined as 4%, which reveals an increase of approximately 20% within a decade (2). Allergen-specific antibodies to milk, peanut and egg have been detected in an estimated 12%, 9% and 7% of US children, respectively (2). Similar trend has been observed in other allergic conditions such as asthma, rhinitis and atopic dermatitis (3). The reasons for the observed increase are yet unknown, regardless of the introduction of the "hygiene hypothesis". The latter lacks sufficient explanation for the immunological changes toward T helper type 2 ($T_h2$)-polarization (4). It is becoming clear that food allergies result from a complex interplay between environmental and genetic factors (5). In addition, the food allergens themselves play an important role as it has been observed that some foods are more allergenic than others (6). The majority of food allergies (about 90%) are caused by milk, egg, peanut, tree nuts, wheat, soybeans, and seafood (5). Differences in the severity of food allergies exist which might be associated with the various allergenicity of food proteins. For instance, cow's milk and egg allergies are the most common food allergies, but they are also more likely to be spontaneously outgrown with time (approximately 85%), while less than 20% of peanut and tree nuts allergies spontaneously resolve, but, on the contrary, are associated with persistency and the majority of life-threatening and fatal anaphylaxis (7, 8). In general food allergic symptoms involve the skin (rash, pruritus, urticarial, etc.), the gastrointestinal (GI) (nausea, vomiting, diarrhea, etc.) and the respiratory tract (dyspnea, tachypnea, laryngeal edema, etc.) (9) and occur within a few minutes up to an hour upon exposure to the allergen. In some cases severe systemic reactions occur. The majority of anaphylactic reactions occurring is caused by food allergens. Food-induced anaphylaxis is estimated to range from 3.2 to 7.6 cases per 100 000 inhabitants per year and this rate is reported to depend on the specific dietary habits of the region (10).

Nowadays, the standard care in food allergy is strict avoidance, nutritional counselling and elimination diets. This "treatment" option is accompanied by the constant preparedness to use antihistamines, corticosteroids and injectable epinephrine to relieve symptoms upon accidental ingestion of the food allergens. Due to the lack of precise diagnostic methods to reveal the optimal tolerated dose of allergen by the patient, the only possibility is that all patients are subjected to strict elimination diets (6, 11). However, even in the most precautious patients frequent accidental exposure to the food allergens occurs (12). This might result from inappropriately declared or undeclared allergens in commercially available products (13, 14), as well as the misinterpretation of labels (15). As a result, the constant fear of accidental ingestion and anaphylaxis limits everyday activities (e.g. day care, school and social events) and imposes stress, uncertainty and heightened anxiety in patients and their families (16). This, on the other hand, affects negatively the quality of life and emphasizes the need for curative therapies. Such therapies will allow patients to tolerate at least a defined amount of the allergic food which will lower or eliminate the risk from allergic reactions upon accidental exposure (17).

### *Basis of immune tolerance*

The intestinal immune system is the largest immune system in the human body (18). In the GI tract, only a single layer of epithelial cell separates the external environment from the intestinal immune system. In the mean time, the GI tract is constantly exposed to an enormous amount of commensal bacteria and food antigens. However, the intestinal immune system possesses the ability to maintain hyporesponsiveness to harmless antigens or microbes, such as food proteins and commensal bacteria, while it maintains the ability to mount vigorous protective responses to pathogens. The state of immunologic hyporesponsiveness to these harmless antigens or microbes is called immune tolerance. The fact that only a small percentage of people suffer from food hyperresponsiveness, even though the exposure to different food antigens is very high, results from the oral tolerance phenomenon. Oral tolerance has been described as the active suppression of systemic immunological responses to an antigen resulting from a prior exposure to this antigen via the oral route (19). Suggested mechanisms of oral tolerance are the induction of deletion or anergy of antigen-specific T cells and the production of regulatory T cells ($T_{regs}$) (20). In addition, factors, such as the route of first exposure, the characteristics of the antigen and the age and genetics of the host, are considered to contribute to the development of oral tolerance (4, 21). However, it is widely hypothesized that the inability to build or defects in the already established oral tolerance result in food allergy.

### *Focus on therapeutic possibilities*

Increasing amount of evidence on oral tolerance mechanisms explains the focus on inducing tolerance to antigens as possible approach to prevent or effectively treat allergies. Allergen-specific immunotherapy (SIT) is one of the most researched approaches to treat allergies by targeting underlying mechanisms of the disorder (22). Its potential of modifying the course of the disease while also managing symptoms has been observed in successful studies for inhalant allergen (e.g. allergic rhinitis, asthma) and venom hypersensitivities where SIT shows to be effective and safe (22, 23). The goal of SIT is inducing tolerance to an antigen by activating cellular and molecular mechanisms by the repeated administration of increasing doses of antigen extracts (24). In contrast to the currently available subcutaneous immunotherapy (SCIT) that is approved for venom and respiratory allergies, such approaches are only on experimental level for food hypersensitivity (25). The possibility of administrating food extracts subcutaneously showed effective in reducing symptoms and lowering allergen-specific IgE levels but also resulted in unacceptable rate of adverse effects and systemic reactions (26). Thus, the scientific interest is shifted to the use recombinant allergens (27) or alternative routes of administration, such as oral (OIT), sublingual (SLIT) or epicutaneous (EPIT) (1, 28). However, information from successful SCIT for allergic rhinitis and bee venom allergy suggests that SIT induces peripheral T cell tolerance by suppressing IgE-mediated histamine release and increasing the threshold for mast cell and basophil activation (17, 29). In food allergy, increasing number of studies with OIT and SLIT contribute to the unravelling of the actual working mechanisms of the SIT, while also testing their efficacy and safety. Most of the SIT therapies studied for food allergy have proven effective; however, they result in numerous unwanted allergic side effects. Novel strategies for improving the safety profile of SIT while preserving its therapeutic effects are highly needed.

Innovation in the field of immunotherapy is focused in improving efficacy and safety of the emerging SIT modalities. Specific strategies, such as peptide immunotherapy that aims at T cell stimulation without IgE cross-linking or additives to SIT that might encourage non-allergic immune response or improve the efficacy and safety of SIT, have attracted the scientific attention (1). Previous *ex vivo* and *in vivo* studies have shown that not only entire proteins are required to induce oral tolerance, but also peptides, such as T cell epitopes present in allergens, can reduce the immunological response to the specific protein (30, 31). Preventive animal studies with hydrolysates, partial rather than extensive, have demonstrated efficacy in reducing symptoms and inducing tolerance (32, 33), but underlying mechanisms and exact tolerogenic fragments within the hydrolysates are still under investigation.

Along with the interest in disease-modifying treatment for food allergies, current research focus is in finding dietary components which beyond their nutritional properties can actively modulate functions in the GI tract and immune responses. Studies with infants have shown the beneficial effect of breast-feeding on tolerance induction that is amongst others contributed to the presence of immune-modulatory non-digestible oligosaccharides abundantly present (the third-largest fraction) in human milk (34, 35). The beneficial effect of those oligosaccharides results not only from enhancing the gut microbiota but also from directly influencing mucosal immune cells (36, 37). Neutral oligosaccharides in human milk have relatively complex composition and their functional properties are yet incompletely understood (38). Isolating human milk oligosaccharides is a very difficult and expensive procedure which hampers their potential use in research. For this reason, synthetic analogues have been manufactured to structurally and functionally resemble some of the human milk oligosaccharides. A specific mixture of non-digestible short-chain galacto- (scGOS) and long-chain fructo-oligosaccharides (lcFOS) in a ratio 9:1 with or without pectin-derived acidic-oligosaccharides (pAOS) has been studied and shown to modulate allergic manifestations *in vivo* by inducing $T_h1$-polarization or $T_{reg}$ cells (37, 39, 40). Broadening the spectrum of treatment possibilities for food allergy by researching adjuvant applicability, combination of therapies or the use of modified allergens increases the chance of developing an effective and safe approach for treatment of food allergy.

This review will focus on cow's milk allergy, being one of the most common allergies, and peanut allergy, being one of the most persistent and severe allergies. The latest findings in tolerance induction and in specific immunotherapy modalities for the two types of food allergy will be reviewed. In addition, the possibilities of combining strategies with other factors to improve efficacy and safety of future treatments will be unravelled.

## Mechanisms of allergy and tolerance

The gastrointestinal track is a very important organ in the human body. It has the difficult task of processing ingested foods and making the possible the absorption of the nutrients in order to facilitate growth and energy production. However, while performing these essential functions, it has to protect from entry of harmful pathogens and an abundance of antigenic proteins and to allow for the gut colonisation with commensal microbes (1, 12). To achieve this, the GI track utilises various physiological and immunological mechanisms. When food is ingested and the food proteins reach the gut, a normal response is the induction of non-responsiveness to the food antigens, also referred to as oral tolerance (41). Some potential mechanisms underlying this oral tolerance are the induction of deletion or anergy of antigen-specific T cells and the production of regulatory T cells (20). Factors, such as the availability of the antigen, the immune environment, the type and activation/maturation status of the antigen-presenting cells (APC) (41), route of first exposure, the characteristics of the antigen and the age, genetics, and intestinal flora of the host, are considered to modulate or interfere with the process of oral tolerance induction (4, 21).

Upon ingestion, food proteins come in contact with the first-line of defence in the GI track, namely the gastrointestinal barrier. This barrier is composed of a single layer of columnar epithelial cells, covered with thick mucus layer. The main function of the barrier is to separate the intestinal immune system from the outside environment and to prevent the entry of harmful pathogens to the systemic circulation. A vital step in processing ingested foods is the break-down of food proteins by gastric acids, enzymes and bile salts, which reduces their immunogenicity due to conformational B cell epitope disruption. Logically, any disturbances in the gastric digestion, such as administration of antacids or anti-ulcer drugs, would contribute to the absorption of intact proteins which increases the risk of developing food allergy (42, 43). Another factors contributing to the increased availability and absorption of intact food proteins, and thus increased risk of sensitization, is the genetically predetermined or pathophysiologically induced increase in intestinal permeability (44).

The second-line defence against pathogens in the GI track is orchestrated by cells of the innate and adaptive immune system which, in addition, play role in oral tolerance induction. Representatives of the innate immune system are intestinal epithelial cells (IEC), dendritic cells (DC), macrophages, neutrophils which can be activated amongst others via their extracellular or intracellular Toll-like receptors (TLRs). Epithelial and dendritic cells play a very important role in oral tolerance induction. The IEC can be classified as non-professional APC and they are able of presenting antigens on surface class II major histocompatibility complexes (MHC class II), but do lack additional co-stimulatory molecules which may render them major players in tolerance induction (1). In *in vitro* co-culture experiments it was shown that IEC express regulatory function by presenting autoantigen directly to T cells and inducing expansion of both CD4+ T effector cells and even more CD4+ regulatory T cells in a MHC class II-dependent manner (45). On the contrary, dendritic cells, which are professional APC, are of great importance as they play a role in the decision of tolerance induction or mounting a robust protective immune response against harmful pathogens by mediating the naïve T cell differentiation. How they control the balance between the two events is not completely known yet. One study suggests that Wnt-betacatenin signalling in intestinal DC is responsible for balance regulation (46). Other studies propose the possibility that

the local microenvironment in the gut, including the cytokine milieu, determines the phenotype of DC (tolerogenic vs. stimulatory) and thus the T cell response to be triggered (47). Once being tolerogenic, DC might implicate several mechanisms to apply this function to T cells, such as secretion of immunosuppressive cytokines, expression of the death-inducing Fas ligand and deficient or adapted co-stimulatory signalling (41, 48). As far as the adaptive immune system is concerned, intraepithelial and lamina propria lymphocytes, Peyer's patches (PP), mesenteric lymph nodes (MLN) and humoral factors such as secretory IgA, and IgG and IgE antibodies are involved in tolerance induction (1). The importance of PP and MLN in generation of proper mucosal immune responses and tolerance to soluble antigens has been supported by *in vivo* studies in which one of the two has been deficient or defective. The reports on PP's importance in tolerance induction, however, have been controversial (41), while MLN has been found to be crucial to tolerance induction as no tolerance occurs when MLN is deficient or defective (49). A key role in oral tolerance and gastrointestinal immune homeostasis is attributed to regulatory T cells that control the immune response via a cell-to-cell contact or by secretion of immunosuppressive cytokines, such as interleukin-10 (IL-10) and TGF-β. A main distinction between $T_{reg}$ cell subtypes is whether they are naturally occurring (produced in the thymus) or they have resulted upon encountering antigen in the periphery (adaptive or induced (*de novo*) $T_{reg}$ cells). Further, natural and *de novo* $T_{reg}$ can be distinguished since they express Foxp3 and are $CD25^{hi}CD127^{low}$, while other $T_{regs}$ like Tr1 and Th3 are classified by their cytokine profile.

Another possible mechanisms for tolerance induction is contributed to the $CD103^{+}$ mucosal DC which are mainly located in the lamina propria, but upon oral administration of antigens traffic to the MLN where they induce *de novo* $Foxp3^{+}$ $T_{reg}$ cells in the presence of retinoic acid and TGF-β (50). An important observation is that once tolerance is established in the intestines, its effects become systemic and this phenomenon is likely facilitated by the MLN which works as a bridge between the mucosal and peripheral immune systems (41).

### *Characteristics of the allergen and the intestinal flora*

Other important factors in tolerance induction and maintenance are the properties and the dose of allergen as well as the intestinal flora condition. Some animal studies have suggested that the dose of antigen, being low or high, determines the underlying mechanisms of tolerance induction, such as $T_{reg}$ cell activation or effector T cell deletion and/or anergy, respectively (20). On the other hand, normal commensal gut flora has been found crucial for normal oral tolerance development as suggested by animal studies with germ-free mice failing to develop tolerance (51). Supporting data on the importance of commensals in prevention of allergic sensitization has been provided from mice with higher rate of peanut sensitization due to the lack of certain toll-like receptor signalling or upon treatment with antibiotics (52). In clinical practice a correlation between atopic dermatitis and disturbed intestinal/stool flora has been observed and the potential of prevention by means of probiotics have been suggested (53), further highlighting the importance of commensal bacteria in the gut homeostasis.

### *Route of sensitization*

It is widely hypothesized that the inability to build or defects in the already established oral tolerance result in food allergy. However, not only a direct disturbance of tolerance induction or breaching existing tolerance might lead to a food allergic disorder, but also bypassing oral tolerance by presenting the antigen via an alternative route, such as the respiratory tract or the skin might result in sensitization and allergy (1). It has been observed that sensitization to pollen via the respiratory tract may result in IgE antibodies able to bind some food proteins (in vegetables or fruits) and subsequently result in the so-called pollen-food-related oral allergy syndrome (1, 54). Increasing amount of evidence suggests that food protein exposure via the skin of susceptible patients might induce sensitization and allergy to a stable protein, especially if oral tolerance has not been previously established (1). This hypothesis has been supported by the results from an animal study where mice were exposed to a food protein directly on the skin and they mounted systemic allergic reactions to the food allergen upon subsequent oral exposure (55). Similarly, in humans it has been found that applying skin creams containing peanut oil to infants with atopic dermatitis is more likely to promote sensitization than peanut consumption of the mother during the pregnancy or lactation periods (1, 56). This observation together with the recent findings that loss-of-function mutations within the *filaggrin* gene (*FLG*), which is associated with development of atopic dermatitis and peanut allergy (54, 57, 58), support the notion that disruptions of the skin barrier might provide an alternative early sensitization route for food allergens.

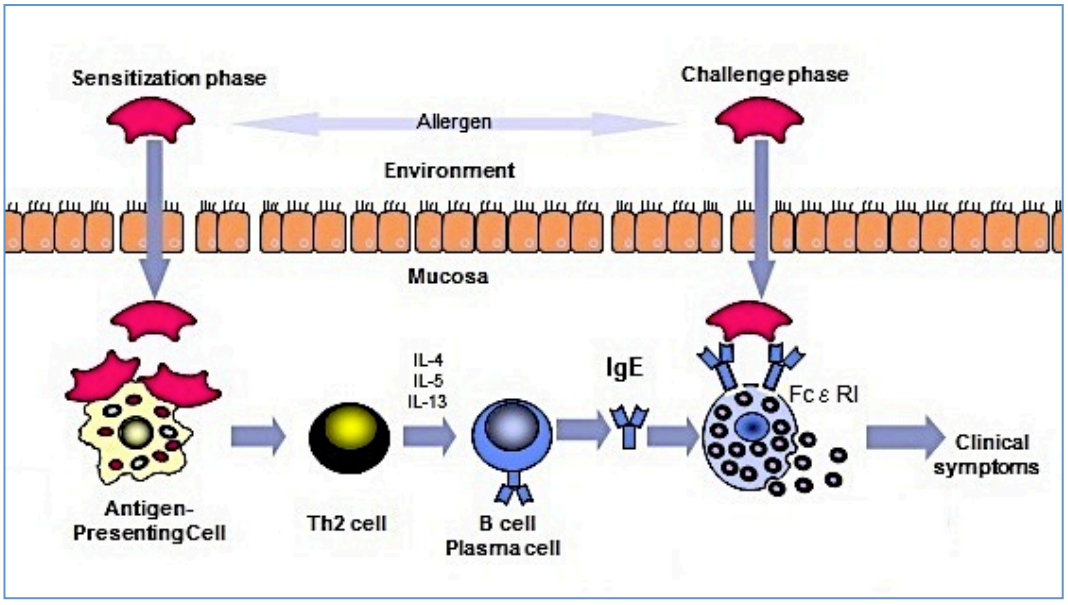


**Figure 1** Mechanisms of allergy. During the sensitization phase allergens are presented by APC to naïve T cells which differentiate into $T_h2$ cells rather than becoming tolerogenic. Due to secretion of IL-4, IL-5

### *IgE-mediated food hypersensitivity*

Food allergy can be subdivided according to its underlying mechanisms. This classification provides three general types of food allergic disorders, namely IgE antibody-dependent, cell-mediated (lacking detectable local and systemic specific IgE levels) and mixed type. A recently suggested mechanism for immediate hypersensitivity-like reactions in the absence of IgE antibodies involves antigen specific activation by means of immunoglobulin free light chains (IgLC) (59, 60). The immediate type IgE-mediated food allergy, also called type-1 hypersensitivity, is responsible for more than 60% of the food allergic reactions and is the focus of this review. Allergen hypersensitivity is normally subdivided in two phases - the sensitization phase and the challenge phase (Figure 1). After food is being ingested, it comes into contact with the intestinal barrier. Food proteins have the ability to cross the barrier and to be exposed to cells of the immune system. The uptake of food proteins and their presentation on the surface of APC facilitates their recognition by a specific T cell receptor, which recognition, if in the presence of IL-4, results in differentiation of naïve antigen-specific T cells into specific $T_h2$ cells in the PP or MLN (41). The effector T cells traffic via the blood stream and home back into the intestinal lamina propria. Once $T_h2$ cell predominance is established, APC (mainly DC) take up the food antigen and stimulate further the clonal expansion of allergen-specific $T_h2$ cells which on their turn produce cytokines such as IL-4, IL-5, and IL-13. The secretion of these cytokines has the ability of inducing the immunoglobulin class switch to the ε heavy chain in B cells, which leads to the production of allergen-specific IgE antibodies. The latter ones are able of binding the high affinity FcεRI which is present on the surface of mast cells, basophils, and APC. Binding of allergen-specific IgE to FcεRI on APC further enhances the uptake of the inoculant allergen (61). At this time point a person is sensitized to the specific food allergen. A consequent exposure to the same allergen (the challenge phase) will then result in the cross-linking of adjacent surface-bound allergen-specific IgE which will activate mast cells and basophils, resulting in their degranulation and release of mediators, such as histamine, leukotrienes, and prostaglandins. Once released those substances mediate the development of type-1 hypersensitivity reactions, symptoms of which are urticarial, angioedema, vomiting, diarrhoea, and anaphylaxis (4).

## Induction of oral tolerance or desensitization

Oral tolerance is the natural way of processing harmless and food antigens upon exposure via the oral route (19). Increasing amount of evidence on natural oral tolerance and its underlying mechanism suggests that inducing tolerance by means of immunotherapy might constitute a curative approach to treat food allergies. Even though the ultimate goal of immunotherapies is the induction of permanent oral tolerance to food antigens, it is still difficult to achieve it in practice. In clinical terminology, oral tolerance is described as the induction of long-term immunologic changes allowing the consumption of the allergenic food without resulting in allergic reactions even after discontinuing the immunotherapy. As there are no immunological markers for tolerance, the permanence of the protective effect achieved with the specific treatment should be tested by intentionally discontinuing the therapy for at least four weeks followed by an oral challenge (62, 63).

An important question is whether effects seen from successful immunotherapy are due to induced permanent tolerance or due to desensitization. Desensitization results in increasing the threshold of antigen needed to induce an allergic response (4). The disadvantage of desensitization is that it requires regular (mainly daily) consumption of the food allergen (so called "maintenance dose") to preserve the achieved threshold. Upon discontinuation of the antigen exposure, the effects are decreased or lost and unwanted allergic reactions occur at much lower antigen doses. This has a tremendous influence on immunotherapy as discontinuation might be needed in cases of illness or viral infections. In addition to this, it has been evaluated that other factors, such as physical exercise, time of dosing, recurrent illness, stress, menses or exacerbated asthma can increase the risk of allergic reaction to a previously tolerated maintenance dose (62). Suggested underlying mechanisms of desensitization include early increase of antigen-specific IgG4 and later decrease in antigen-specific IgE antibody levels, accompanied by decrease in activation of mast cells and basophils and transient increase of antigen-specific $T_{reg}$ cells (62, 64). In the majority of clinical studies conducted, it is desensitization that has been reported rather than tolerance. However, an important question is whether it is a matter of prolonged desensitization to achieve tolerance or whether other tolerance induction pathways are needed.

## Antigen-specific immunotherapy for food allergy

Nowadays, the only available and standard method for managing food allergies is strict avoidance of the allergenic food. However, due to the nutritionally and socially limiting character of this approach, the need for disease-modifying management of food allergies has shifted the scientific interest toward allergen-specific immunotherapy as most highly potential curative approach. It focuses on exposing patients to gradually increasing doses of allergen via various routes, such as subcutaneous, oral, sublingual or epicutaneous. The aim of immunotherapy is to modulate the response to food allergens while preventing any adverse events that might result from the therapy itself (1). The ultimate goal of the approach is achieving tolerance to the specific allergen which will allow consumption of the food without allergic reactions even after discontinuation of the successfully achieved maintenance dose.

In the clinical setting SIT has been performed already for 100 years (24). However, its clinical application for food allergy has not achieved an approval yet, lagging far behind SIT for other allergies, such as allergic rhinitis, bee venom allergy and drug allergy (17). Efficacy of tolerance induction methods to food allergens has been reported since 1829 when Dakin described how Native Americans were fed poison ivy leaves to prevent contact hypersensitivity to urushiol, the sensitizing antigen in poison ivy (65). In another setting, guinea pigs were protected from anaphylaxis by means of prior oral exposure to hen's egg protein (66). Successful immunotherapy would render beneficial for the quality of life of food allergic patients and their families; however, a downside of immunotherapy approaches is that they will require to be specifically designed for the different food allergens.

### *Subcutaneous immunotherapy*

Standard allergen-specific SIT is administered via the subcutaneous route (SCIT). This modality has proven effective and safe and is a well-established practice for respiratory and bee venom allergies (54). In food hypersensitivities, records of using SCIT for fish allergy dates back to 1930 when Freeman administered cod fish juices subcutaneously to a boy, followed by a fish diet involving daily ingestion of cod liver oil (67). SCIT has been tested 20 years ago also for peanut allergy by means of injecting aqueous peanut extract (68). During the study around 67-100% efficacy in modulating the immune response, reported as decrease in allergic symptoms, after a double-blind, placebo-controlled food challenge (DBPCFC) was reported. In addition, all three subjects who completed the treatment showed reduced skin prick test reactivity to peanut extract compared to one placebo-treated subject who did not show any changes in the above-mentioned parameters. Unfortunately, this efficacy was accompanied by frequent systemic reactions to the therapy and even a tragic outcome for one control subject who died after receiving a dose of peanut extract due to a pharmacy error (68). This event resulted in the termination of the study and important conclusions were made about the serious risks accompanying food immunotherapy via the subcutaneous route.

In a follow-up study from the same group, 12 patients with peanut hypersensitivity were recruited and 6 were treated with peanut extract SCIT and the other 6 were followed as untreated controls (26). The treatment group was subjected to a rush immunisation protocol in order to achieve a maintenance dose which was then administered weekly for the period of 1 year. All subjects in the study underwent

DBPCFC at the end of the study and showed increased tolerance to oral peanut challenge together with reduced sensitivity to peanut extract measured by skin prick test. However, three of the subjects in the treatment group needed a reduction of the previously achieved maintenance dose due to adverse reactions. This reduction led to a partial or complete loss of protection to peanut challenge. The treatment effect observed in the SCIT subjects was accompanied by increased peanut-specific IgG levels, but no differences in IgE levels were detected. In contrast, the untreated control group showed no improvements in the parameters. Despite the clinical efficacy detected in this study, the rate of systemic adverse reactions to the therapy was unfavourably high both during the rush protocol (23%) and during the maintenance immunotherapy (39%) (26).

The clinical experience with SCIT in inhalant allergen sensitivities creates a lot of expectations for the possible applicability and efficacy of SCIT in food hypersensitivity. Studies with effective SCIT in inhalant allergies have suggested that suppression of the recruitment of effector cells occurs, together with basophil and eosinophil reduction in numbers and increase of allergen threshold for mediator release (22). In addition, induction of $T_{reg}$ cells takes place, taking care for the induction of peripheral tolerance to the allergen by inhibiting immune responses to it (22). Thus, the increasing amount of evidence about the working mechanisms of SCIT in respiratory allergies provides information about potential biomarkers which could be advantageous for predicting efficacy and monitoring safety in other allergic conditions as well. In addition, this route of administration requires relatively small amounts of allergen compared with other routes. Although the two aforementioned studies represent a proof of concept for the use of food allergen extracts in SCIT, the significant rate of adverse reactions renders this treatment modality currently unfavourable and discourages further studies unless improvements in the form of the administered allergen are introduced.

### *Oral immunotherapy*

Due to the lack of success of SCIT in food hypersensitivity, the scientific community have gained interest in oral immunotherapy and sublingual immunotherapy as safer and less invasive alternatives. OIT is so far the most actively investigated form of immunotherapy for food allergies. In OIT, patients are given gradually increasing amounts of powdered food protein mixed with a food vehicle (e.g. apple sauce) until a target dose is achieved. This normally takes place in a monitored setting. Once the target dose is reached, patients are subjected to daily regular ingestion of the tolerated dose at home during the maintenance phase (28, 54). The initial aim of OIT studies is to show desensitization, hopefully followed by tolerance induction with time. In order to detect any improvements contributed to OIT, study groups very often perform DBPCFC in the beginning and in the end of OIT protocols (54). In this review it will be focused on OIT for cow's milk allergy and peanut allergy due to the frequent occurrence of the former and the severity and persistence of the latter.

#### *Cow's milk OIT*

Records on OIT date back to 1908 when an egg-induced anaphylaxis in a child was successfully managed with OIT (69). In the beginning, there were mostly uncontrolled studies of OIT for different food allergens, including cow's milk. In the 1980s and 1990s Patriarca et al. showed that OIT could effectively be used in cow's milk allergy (70, 71). In a follow-up study, evaluating OIT for cow's milk, egg, and fish allergy, they showed

that desensitization was achieved in 83% of the enrolled food allergic patients, when compared to age-matched controls for the various allergens (72). In addition to evaluating efficacy, this study was the first to evaluate any immunologic changes resulting from successful OIT. After 18 months, the investigators reported decreased levels of allergen-specific IgE and increased allergen-specific IgG4 accompanied by bringing skin prick test responses to negative. Those findings encouraged further OIT research in food allergy because of the similarities between the immunological changes usingOIT for food allergy compared to standard subcutaneous immunotherapy for respiratory allergies (54).

Another two very important studies on cow's milk OIT are the prospective study performed by Meglio *et al.* in 2004 and another study by by Staden *et al.* in 2007. After a long desensitization protocol, Meglio *et al.* evaluated the efficacy of OIT in 21 children aged between 6 and 10 with IgE-mediated cow's milk allergy and reported an increased amount of milk that could be tolerate per day (200ml) (73). In addition, the children were followed and the persistence of the achieved effect was studied. Surprisingly, after 5 years 9 of the 16 treated children who completed the study were still tolerant. In the second study, Staden *et al.* compared OIT versus elimination diet in subjects aged between 0.6 and 13 years suffering from cow's milk (n=14) or egg allergies (n=11) (74). As this was the first study to evaluate the permanence of desensitization achieved by OIT, all subjects had to strictly avoid the food allergens for 2 months after completion of the induction phase. Following the avoidance phase, all patients underwent food challenge and 36% of the treatment group showed persistent tolerance; however, 7 of the 20 control subjects (35%) appeared to have spontaneous resolution of the allergy. Unfortunately, 9 patients did not finish the study due to persistent side effects. Having these two studies in mind, it is very difficult to make any conclusions about the permanence of the desensitization achieved with OIT due to the transient nature of cow's milk allergy (75).

Up to that moment, the performed studies involved cow's milk allergic patients, but not ones with severe allergic responses due to the uncertainty of the OIT safety. In 2008 Longo *et al.* conducted the first randomized placebo-controlled clinical trial with cow's milk allergic children (aged between 5 and 17) with previous history of cow's milk-induced anaphylactic reactions (76). The recruited patients were randomly assigned to OIT or elimination diet. The beginning of the study was performed in a hospital and later maintenance was continued at home. During the in-hospital build-up day children were given an initial dose of 0.4mg milk protein with a regular escalation aiming at a final dose of 50mg. this was followed by daily home dosing with 8 weekly in-hospital dose escalations with the aim dose of 500mg. Once 500mg was reached, children were subjected to this dose daily during the 3-4 month maintenance phase. After completion of the study, 36% of the treated group managed to tolerate at least 150ml cow's milk, while 54% could tolerate amounts between 5-150ml. On the contrary, control subjects on elimination diet could barely tolerate 5ml cow's milk. Looking at the important aspect of safety, only 10% (3 patients) needed to drop the study due to persistent abdominal and respiratory complaints. However, mild adverse effects, such as urticaria, angioedema and abdominal pain, were relatively common in the treatment group. Despite the common side-effects, which are not surprising keeping in mind the specific

**Table 1 Summary of several studies on cow's milk oral immunotherapy**

| Study | Setting | Patients N | Time to maintenance | Results | Comments |
|---|---|---|---|---|---|
| Patriarca *et al.* **(72)** | Open controlled, nonrandomized Control group on elimination diet | Total: 59 patients 29 with CMA 15 with egg allergy 11 with fish allergy 4 with other allergies 16 in control group age 5-55 years | 4 months | 83.3% desensitization; 77.8% showed decreased SPT after 18 months | In the CMA patients, severe reactions resulted in 5 subjects dropping out of the study. Overall, around 51.1% of subjects experiences side-effects (e.g. urticaria, angio-oedema or abdominal pain). |
| Meglio *et al*. **(73)** | Prospective uncontrolled | 21 in active group no control group age 6-10 years | 6 months | 71.4% fully tolerant; 14.3% partial tolerance; 14.3% | After 4 years, 9 patients were completely tolerant. No clear conclusions due to possible spontaneous resolution. One subject regained allergy after 6 months of therapy and few weeks off therapy due to illness |
| Longo *et al.* **(76)** | Randomized placebo-controlled Control group on elimination diet | 30 in active group 30 in control group age 5-17 years | 10 days (hospital) + 3 months (home) | 36% tolerated 150ml CM; 54% tolerated 5-150ml CM | First study with CM allergic patients with history of anaphylaxis to CM. 10% (3 subjects) dropped out of the study because of systemic reactions. 17/30 (57%) from the active group reported side-effects at home. |
| Skripak *et al.* **(77)** | Randomized Double-blind Placebo-controlled | 13 in active group 7 in control group age 6-21 years | 23 weeks | 92% of active group tolerated median dose of 5140mg CM; no change in placebo group | Increased tolerated dose in the active group only; decreased end point SPT; no milk-specific IgE changes, but milk-specific IgG4 was markedly increased. Side-effects median frequency was 35% in active group vs. 1% in placebo group. |
| Staden *et al.* **(74)** | Randomized open controlled | 14 CM and 11 egg patients; 20 in control group age 0.6-12.9 years | 67 days | 35% permanent tolerance; 12% desensitization; 16% partial responders | First attempt to test the persistence of induced tolerance by discontinuing OIT for 2 months. Spontaneous resolution of CM allergy was reported in 35% of the control group (similar to the % efficacy in the active group). |
| Pajno *et al.* **(78)** | Randomized Single-blind Control = soy milk | 15 in active group 15 in control group age 4-13 years | 18 weeks | 76% of active group achieved 200ml CM; no improvement in placebo group | First trial with adapted protocol for weekly up-dosing performed within 18 weeks. 15% (2 children) dropped out of the study due to systemic reaction. |

CMA, cow's milk allergy; SPT, skin prick testing

subgroup of patients recruited, this study provides evidence for the possibility of using OIT in patients with severe food allergies (54).

Later in 2008 the results from the first double-blind placebo-controlled clinical trial for testing OIT in cow's milk allergic patients were presented (77). In this study, Skripak *et al*. randomly assigned 20 cow's milk patients to treatment (13 patients) or placebo (7 patients) and showed that, after successful completion of the study, 92% of the treatment group could tolerate a median dose of 5140mg cow's milk, compared to the control subjects who did not tolerated above the baseline 40mg. The frequency of adverse events in the treatment group was noticeably higher with 35% vs 1% in the control group. In addition, immunological changes, such as decreased skip prick test reactivity and increased cow's milk-specific IgG4, were reported while allergen-specific IgE remained unchanged.

A variety of OIT protocols has been tested, including one with weekly outpatient-increasing doses of active treatment or soy-milk as placebo. In their study, Pajno *et al.* showed that the active treatment was effective in 10 out of 13 patients (aged between 4 and 13) who managed to tolerate 200ml cow's milk (78). In this study, there was also one patient with partial tolerance compared to lack of changes in the placebo group (78). As far as the safety of the treatment was concerned, only 2 patients needed to discontinue the study because of systemic reactions. When monitoring for any immunological changes occurring during the trial, similar to other studies, a significant increase in allergen-specific IgG4 was found, but no differences in specific IgE levels were detected.

*Peanut OIT*

In contrast to common allergies, such as cow's milk, hen's egg and wheat, which have a high percentage of spontaneous resolution (approximately 85%), peanut hypersensitivity is outgrown in less that 15-20%. In addition, patients allergic to peanut are more likely to experience severe reactions upon accidental ingestion of the allergen. In one survey approximately 20% of the anaphylactic reactions triggered by food allergens were contributed to peanuts compared to 14% contributed to cow's milk (79). It is not surprising that accidental exposure to peanuts is one of the most common causes of fatal or near-fatal anaphylactic reactions (80). Successful desensitization via the oral route has already been reported for peanut allergy as well, despite the fewer number of studies exploring the clinical responses and immunological changes following OIT compared to cow's milk allergy (54). After 2009, more trials have been reported on the use of OIT in peanut allergy, starting with a small study with 4 patients who underwent successful desensitization and managed to tolerate between 10-12 peanuts after DBPCFC (81).

Encouraging results were delivered by a larger open-label study with 39 paediatric patients (median age of 57.5 months) with peanut allergy (82). The enrolled patients underwent initial in-hospital day escalation with a starting dose of 0.1mg peanut protein and which dose was doubled every 30min. A build-up phase followed and comprised daily dose intake at home with an escalation of 25mg peanut protein every two weeks until a dose of 300mg was achieved. Once the dose was reached, patients were subjected to 300mg peanut protein daily during the maintenance period. The last phase, the maintenance phase, lasted for several months and was followed by an oral food challenge in order to assess desensitization and which resulted in 74% of the enrolled subjects completing the study. From those who completed the trial 93% successfully tolerated up to 16 peanuts while the rest tolerated up to 9 peanuts. Interestingly, monitoring of immunological parameters throughout the study revealed reduced SPT reactivity and basophil and mast cell activation, accompanied by increased allergen-specific IgG4 and decreased allergen-specific IgE. Novel to this study is the finding that 6-day stimulation of PBMC with peanut resulted in elevated peanut-specific $Foxp3^+$ $T_{reg}$ cell levels during the first 12 months, followed by a decrease thereafter. Additional changes were observed in cytokine production by PBMC, where IL-10, IL-5, IFN-$\gamma$ and TNF-$\alpha$ were increased over a period of 6-12 months (82). The safety report from this trial revealed that side-effects occurred mostly during the initial escalation day at the hospital with 20 out of 28 patients receiving some form of treatment and 4 of those 28 receiving epinephrine (83). Although during the build-up and maintenance phase the

frequency of adverse events was much lower, mild reactions still occurred but did not prevent the subjects from completing the study. However, closer look at the safety of OIT during home-dosing revealed several factors rendering patients were more likely to experience allergic reactions to previously tolerated maintenance dose. Those factors include administering the dose on empty stomach or during menses, exercising after dosing, and dose administration accompanied by illness or suboptimally controlled asthma (84). Further, 8 patients who completed the open-label study and who had reduced peanut-specific IgE levels under 15kU/l were selected for an oral food challenge following 4 weeks off treatment in order to evaluate tolerance induction (85). All subjects passed the food challenge, which together with their laboratory outcomes support the notion of successful tolerance development.

In another study in the same year, Blumchen *et al.* investigated the possibility of inducing tolerance by peanut OIT (80). They subjected 23 patients with peanut allergy to a weeklong in-hospital rush desensitization protocol with a target dose of 500mg whole peanut. Patients were given crushed roasted peanut 2-4 times a day until they reach 500mg of whole peanut or until the one week period was over. Patients, who successfully reached the target dose, continued straight with the 2-month long maintenance phase. On the other hand, the ones, who failed in tolerating 500mg whole peanut during the rush protocol, were subjected to a median of 7-month long build-up phase at home until they could tolerate 500mg in order to proceed to the maintenance phase. 14 of these 23 patients (61%) successfully reached the aimed maintenance dose. The OIT was then discontinued for 2 weeks before DBPCFC was performed. At baseline, subjects tolerated less than 0.19g peanut and had a median peanut-specific IgE of 95.6kU/L. During the oral food challenge after being 2 weeks off-therapy, all 14 patients had a 4-fold increase in tolerated amount of peanut (median 1g peanut) compared to baseline. Three of the 14 subjects could tolerate even up to 4g peanut without allergic reactions. Similar to previous findings, peanut-specific IgG4 levels were increased after OIT together with a decrease in IL-5, IL-4 and IL-2 production from peripheral blood mononuclear cells (PBMC). Interestingly, those findings were less convincing after 2 weeks off-therapy (80). Subsequently, it could be concluded from the aforementioned two studies that more persistent desensitization or even tolerance can be induced in patients with peanut allergy by means of OIT. However, whether OIT effects will be maintained after longer off-therapy periods (>4 weeks) still needs to be investigated.

Not much later, reports from the first randomized double-blind placebo-controlled clinical trial were available in which 28 peanut-allergic children ages 1 to 16 years were involved in studying peanut OIT (86). The active group (n=19) and the control group (n=9) were subjected to an initial, build-up and maintenance phases with OIT containing peanut flour or placebo respectively. The target dose of the initial escalation day was 6mg peanut protein which was achieved by increasing the initial dose of 0.1mg every 30min. The build-up phase (approximately 44 weeks) was performed at home with regular hospital visits for dose escalation every 2 weeks until the target dose of 4000mg was achieved. Once reaching the maintenance dose of 4000mg peanut protein, patients were ingesting it daily for a period of one month. After approximately one year of OIT, DBPCFC revealed that 16 out of 19 patients (84%) could tolerate up to 5000mg (equivalent to 20 peanuts), while control subjects tolerated a median of 280mg of peanut. During the study three patients (15.8%) from the active group dropped out of the study because of allergic reactions. Interestingly, after the DBPCFC only one subject

from the peanut OIT group, compared to 8 patients from the placebo group, developed mild clinically relevant symptoms. This study, just like the ones preceding it, has investigated the concurrent immunological changes related to peanut OIT. Surprisingly, it was found that peanut-specific IgE levels initially increased in the OIT group, but at the time of the final oral challenge they were back at baseline. Confirming findings from previous peanut OIT studies, it was reported that allergen-specific IgG4 levels increased, while the SPT wheel size decreased. Further, IL-5 and IL-13 production by PBMC was reduced, suggesting a shift away from $T_h2$ phenotype, while the increased $Foxp3^{hi}$ : $Foxp3^{intermediate}$ $T_{reg}$ cells ratio could explain the suppressed allergic immune response seen (4, 86).

Taking into account the aforementioned studies, it is evident that OIT is effective in inducing desensitization in food allergic patients. Its main advantage, compared to previously described SCIT, is that it is much safer. In addition, OIT comprises a more convenient route of administration which would allow home dosing and reduce the need to visit the clinic on a regular bases. Another very important advantage of OIT is that it actually makes use of the cells and immune pathways involved in oral tolerance induction (62). Further, it has been observed in animal studies that the dose of the antigen determines the immune response; thus high-dose allergen administration results in deletion or anergy of allergen-specific T cells, while low doses of allergen stimulate suppressive response by means of induction of $T_{reg}$ cells (20, 62). This finding would suggest that OIT might be able to adapt according to the desired immunologic outcome. On the contrary, OIT makes use of much higher allergen doses compared with other SIT modalities which increase the concern of inducing allergic side-effects. The convenience of OIT administration at home can also impose a safety concern because of the possible adverse events taking place in the absence of medical supervision (54). This emphasizes the need to improve not only the efficacy of OIT, so that real tolerance can be induced, but also safety, both of which might benefit from the use of proper adjuvants to the therapy.

### *Sublingual immunotherapy*

Studies for evaluating SLIT as an option of modulating food allergy are much less extensive than those for OIT. However, the interest in the safe and convenient nature of this approach has encouraged several studies to examine the effectiveness of this modality for treating food hypersensitivities. In SLIT, a small amount of liquid concentrated allergen extract (normally in the scope of micrograms to milligrams) is administered under the tongue where it is kept for a certain time period and then spit or swallowed. Similar to other SIT modalities, an increasing amount of the allergen extract is administered during a build-up phase followed by a maintenance phase. However, in contrast to OIT, doses in SLIT begin at micrograms and do not exceed milligrams due to the maximum concentrations of available extracts (87). Success with this form of immunotherapy has been shown in inhalant allergies where sublingual tablets Grazax are currently available for managing grass pollen allergy. The first attempt to apply SLIT in food allergy is in the case of a 29-year-old woman with severe kiwi allergy (88). It was shown that sublingual application of diluted kiwi pulp for 1min followed by swallowing it and after a 5-year maintenance, she tolerated kiwi consumption even after 4-month off therapy. Interesting results on SLIT efficacy have been obtained in clinical studies

with other food allergens, such as hazelnut (89) and peach (90), summarized in a recent review (87).

As far as cow's milk and peanut allergies are concerned, several studies have been dedicated to investigate SLIT, compared to the numerous studies investigating OIT for these allergies. In a small open-label pilot study, a French team treated 8 children for 6 months with cow's milk extract which was hold under the tongue for 2min and then spit out (91). All the seven subjects that completed the study passed a food challenge in which the median threshold increased from 39ml at baseline to 143ml post-treatment. On one side no changes in cow's milk-specific IgE were detected, but on the other side only one subject dropped out of the study due to persistent oral side-effects emphasizing the encouraging safety profile of SLIT.

In a later exploratory open-label study, the efficacy and safety of SLIT for cow's milk allergy has been tested and compared to OIT (92). For this purpose 30 patients with cow's milk allergy were subjected to an initial SLIT escalation up to 3.7mg milk protein and after completion were randomly assigned to continue SLIT up-dosing up to 7mg milk protein daily or to continue with OIT either to 1g (OITB group) or to 2g (OITA group) of milk protein. Efficacy of the different treatments was evaluated by means of DBPCFC after 12 and by means of open food challenge after 60 weeks of maintenance period. The subjects who successfully completed the food challenge after 60 weeks without developing treatment-needing reactions were selected to undergo an elimination diet with challenges after 1 and 6 weeks in order to investigate the permanence of the desensitization. Results from this study revealed that SLIT followed by OIT is more effective in desensitizing patients, but was associated with higher rate of side effects and higher risk of systemic reactions. In the post-treatment challenge only 1 out of 10 subjects in the SLIT-only group tolerated the full challenge amount compared to 6/10 in the OITB and 8/10 in the OITA groups. Only 1 patient from the SLIT group, compared to total of 8 patients in the OIT groups preserved the induced tolerance effect after 6 weeks off-therapy. On mechanistic level, all three groups had increased allergen-specific IgG4 levels and decreased SPT reactivity when compared to baseline. However, milk-specific IgE levels and spontaneous basophil histamine release decreased only in the OIT groups, but not in the SLIT group emphasizing the higher desensitizing efficacy of OIT (92).

From safety perspective, SLIT revealed to be safer approach as it was reported to induce lower risk of systemic reactions, compared to OIT. Even though higher percentage of the SLIT doses were accompanied by side-effects compared to OIT (29% vs. 23%), OIT was significantly more often associated with multisystem, upper and lower respiratory tract, and gastrointestinal adverse reactions than SLIT. Patients who underwent OIT showed higher need for antihistamines and β-agonists than SLIT patients. It is hypothesized that the better efficacy of OIT and the higher rate of side-effects might be contributed to the much higher doses of allergen administered with this therapy approach (about 140-fold higher than in SLIT) (92). In short, OIT appears to be more effective at desensitizing allergic patients, but also less safe than SLIT; however, combining different treatment modalities might prove advantageous but more research is needed to determine the optimal use.

Recently, successful desensitization has been reported in patients with IgE-mediated peanut hypersensitivity as well (93). In this double-blind placebo-controlled clinical trial, Kim *et al.* recruited 18 patients with peanut allergy to be randomly assigned to peanut SLIT (11 subjects) or placebo (7 subjects). Their protocol consisted of peanut extract administration under the tongue for 2min followed by swallowing during a 6-month build-up phase, followed by 6-month maintenance phase. A daily dose of 2000μg of peanut protein was administered during maintenance and DBPCFC was performed after completing the 12-month study. During the challenge, the active group showed an increased reaction threshold compared to the placebo group with a median of 1710mg vs. 85mg peanut protein respectively. Additionally, the percentage of SLIT doses causing adverse reactions was not much higher than the percentage of OIT doses (11.5% vs 8.6% respectively). The majority of the reactions were transient with only 0.3% of home doses requiring antihistamines. Throughout the whole study there was no need of administering epinephrine and most of the side-effects constituted lip swelling, throat itching, finger swelling, pruritus and wheezing (4, 93). Taken together, these data highlight the favourable safety profile of SLIT in treating peanut allergy.

Similar to other studies, Kim *et al.* also monitored the concurrent immunological changes during peanut SLIT. They found significantly reduced basophil activation upon peanut extract stimulation and significantly lower IL-5 levels after 12 months of therapy compared to placebo. Further, mast cells reactivity, measured as SPT reactivity, was significantly decreased in the active group. Allergen-specific IgE levels were found to increase during the first 4 months, but decreased significantly during the rest of the study. Increase in the peanut-specific IgG4 and the percentage of $T_{reg}$ cells were reported in the active group with the latter not to being statistically significant. In contrast, no changes have been detected in the placebo group (93).

Besides the lower frequency of systemic adverse effects, intrinsic tolerogenic properties of the oral cavity and mucosa also contribute to the advantageous profile of sublingual allergen administration. The oral cavity is highly colonized by bacteria but no acute inflammatory processes take place, possibly due to the lack of inflammatory cells. In addition, oral mucosa tissue has wound-healing capacity without any scar development (61). A proposed advantage of the oral tissue is also its high permeability and direct absorption in the circulation without undergoing first-pass metabolism in the liver (4). However, pharmacodynamics studies on allergen trafficking have suggested that some allergens stay in the oral mucosa for several hours (94) and that the increased interaction with it was suggested to enhance efficacy in mice (95). The relatively low numbers of effector immune cells in the oral mucosa, compared to other mucosal sites and the skin, implies that allergens are administered at a non-inflammatory environment; however, presence of mast cells has been detected and they are the potential mediators of the local adverse effects observed with SLIT (96). The pro-tolerogenic properties of the local APC (namely Langerhans cells) together with possible contribution from epithelial cells, secretory IgA and the non-pathogenic resident organisms represent a high potential for tolerance induction (96). This, together with small allergen doses and good safety profile, supports the possibility of SLIT being a desirable immunotherapy approach for clinical practice.

### *Epicutaneous immunotherapy*

An attractive approach for SIT, which has recently been studied, is the epicutaneous one. It utilises a skin patch containing soluble allergen that is absorbed into the stratum corneum. The epicutaneous immunotherapy (EPIT) is an attractive non-invasive and possibly safer way of administrating allergen which has shown promising results in animal studies using peanut-sensitized mice (97, 98). In this study, the novel approach was compared to SCIT and control and revealed to be as efficient as SCIT with the ability to significantly reduce allergen-specific IgE/IgG2a and IgG1/IgG2a ratios. The enthusiasm about this route of administration led to a 3-month long pilot study with 18 children suffering from cow's milk allergy. In this double-blind placebo-controlled clinical trial, patients were randomly assigned to EPIT with a patch containing either cow's milk powder or placebo (glucose) (99). In the end of the study, patients from the active group had an increase in the maximal tolerated dose to a mean of 23.61ml milk compared to the 1,77ml tolerated at baseline. In contrast, no changes in the cumulative tolerated dose were observed in the placebo group.

There are currently no official clinical results available for the use of EPIT in peanut allergy. However, two trials are running at the moment: a phase I trial in the USA is investigating EPIT safety and tolerability in children and adults with established peanut hypersensitivity and a phase II DBPC trial in France is evaluating the proportion of patients that will tolerate 1g peanut at 6 months or have at least a 10-fold increase in tolerated dose compared to baseline(100).

The goal of EPIT is to administer allergen in a safe manner to the outermost layer of the skin, the epidermis. Due to its interesting characteristics (e.g. nonvascularized, multi-layered epithelium which is under the immunological surveillance of keratinocytes and Langerhans cells) the epidermis appears to be a promising route for antigen administration. When antigen is taken up by skin dendritic cells, it is presented in the local draining lymph nodes and associated with induction of systemic IgM, IgG and mucosal IgA responses (101). Additionally, Langerhans cells in the epidermis point at the presence of a crucial pathway for antigen uptake and stimulation of downstream pathways inducing T cell responses (102). The non-invasiveness of this approach and its potential of delivering allergens in a safer manner, circumventing potential induction of systemic immune reactions, render it an attractive option for future therapeutics.

## Mechanisms of allergen-specific immunotherapy

Currently the underlying mechanisms by which SIT in food allergy modulates the allergic immune response and reduces symptoms are not yet clear and well understood. With the increasing number of clinical trials, an emphasis has been put on the concurrent immunological changes in order to shed more light on the mechanisms of clinical protection. The advantage of discovering the mode of action of SIT is that knowing which are the most important and clinically relevant elements in the pathway would enable the development of immunotherapy with enhanced efficacy and minimal adverse effects (103). As noticed in the previously discussed clinical studies, it appears that despite of the route of administration, similar effects have been reported for T cell and antibody responses. Additionally, it has been suggested that the immunological changes occurring with SIT for food allergy are similar to changes reported for SIT in inhalant hypersensitivities (17). Due to the fact that the exact mechanisms of SIT in food allergy are still under investigation, data from successful allergen-specific immunotherapy in respiratory allergies will be used as well to propose possible immunologic mechanisms for food allergy SIT.

### *Effects on allergen-specific antibodies*

In most of the OIT and SLIT studies for food allergies variable effects have been observed on antigen-specific IgE levels. In many cases though, similarly to SIT for inhalant allergens, a transient increase in specific IgE levels is observed followed by return to baseline levels during maintenance or even lower than baseline at the end of therapy. As this late decrease in allergen-specific IgE levels does not correlate with the clinical protection seen earlier in the course of SIT, it is more probable that other mechanisms underlie the early reduction in allergic symptoms. Interestingly, in the majority of human studies with SIT for food allergy, in either route of administration, a significant increase in specific IgG antibodies (mainly IgG4) has been reported (77, 78, 82, 89). IgG4 is a non-inflammatory and non-complement isotype and thus considered protective in allergic reactions. It is suggested to exert its protective function by disturbing the binding of allergen to effector cell-bound IgE and thus supressing mast cell and basophil activation (29, 61). IgG4 is speculated to prevent IgE-allergen interaction by capturing allergens, even if it is directed to epitopes others than IgE-binding ones. This specific blocking activity of the IgG4 isotype appears to be more important than IgG4 levels, because IgG4 levels show correlation to the administered SIT dose rather than to the clinical improvement observed (96, 103, 104). An *in vitro* assay has been introduced to measure the functional activity and the affinity of blocking antibodies induced by SIT (105). With the use of this technique data is obtained suggesting that successful SIT might enhance IgG-blocking activity independently of enhancing its quantity. In addition, a correlation between the inhibitory potential of IgG and the size of the allergen-IgG complex has been proposed, with lager complexes possessing higher blocking potential (29). An additional suggestion for the effectiveness of allergen-specific IgG comes from the findings that non-allergic beekeepers have a much higher allergen-specific IgG4/IgE ratio than bee venom allergic patients (106). Interestingly, an increase in the specific IgG4/IgE ratio caused by allergen-specific immunotherapy might be indicative of a T cell response shifting from an allergen-specific $T_h2$ to $T_{reg}$ response. Apparently, IL-10 together with IL-4 is needed to induce class-switching to IgG4, which implies that increased IL-10 levels during SIT would

suppress IL-4-induced IgE switching, but will stimulate IL-4-mediated IgG4 production (17, 29, 61). This sheds light on the dual role of IL-10, which, next to inducing T cell tolerance, skews the immune response to a non-inflammatory phenotype.

Recently, some more attention has been granted to the induction of other antibody isotypes with protective potential, such as allergen-specific IgA. In human and animal studies with SIT administered via the sublingual or oral route, increased specific IgA levels have been detected in saliva (106) and in serum (107), respectively. This increase in allergen-specific IgA levels correlated with SIT efficacy and suggestions on its immune relevance point at the ability to neutralize absorbed antigens in the circulation next to preventing antigen uptake upon ingestion (108). Further research is required to confirm the importance and the exact role of antibodies, such as IgG, IgG4 and IgA, in allergen-specific immunotherapy and tolerance induction to food allergens.

### *Effects on effector mast cells and basophils*

One of the main goals of SIT is to modulate the threshold for mast cell and basophil activation and to prevent unwanted IgE-mediated release of mediators upon accidental ingestion of the allergen (29). Subcutaneous immunotherapy for inhalant allergies, for example, reduces the numbers of effector cells in mucosal sites and diminishes their reactivity *in vitro* (96). From data obtained during the different clinical trials with food immunotherapy, it is controversial whether SIT induces allergic effector cell hyporesponsiveness due to the various results on skin prick test reactivity and *in vitro* basophil activation studies. However, data from SCIT for bee venom allergy and data from desensitization studies with drug allergy have revealed an early decrease in degranulation activity of mast cells and basophils (17, 29). It has been observed that in mice undergoing acute oral desensitization, this initial effect on mast cells is contributed to mast cell desensitization (109). It is suggested that a constant release of under-activation-threshold levels of mediators, such as histamine and leukotrienes, occurs soon after SIT initiation and somehow renders mast cells and basophils less responsive. The small amounts of mediators, which are below the threshold for inducing systemic anaphylaxis, might contribute to the reduction of granule content as well (17, 29).

In food immunotherapy, several studies have reported reduction in mast cell and basophil activation (82, 92, 93). In most of the results, the skin prick test, measuring mast cell reactivity, correlated with the results from *in vitro* stimulation of peripheral blood basophils with food allergens. However, SPT appeared to be with slightly higher sensitivity than basophil histamine release measurement, suggesting that additional markers might be more proper to monitor, such as constitutive expression of CD63 and CD203c on basophils which was decreased during immunotherapy. Additional observations on CD63 and CD203c expression suggest that patients with higher increase of basophil activation marker expression in the beginning of immunotherapy have poorer outcome in the late course of the therapy (92). This conclusion might be helpful to monitor and possibly predict clinical response. Changes in other immune parameters, such as allergen-specific IgE levels and $T_{reg}$ cells, potentially contribute to the decreased mast cell and basophil degranulation as well. However, more and larger studies are required to further elucidate the effect of food allergen specific immunotherapy on mast cells and basophils and their exact contribution to the induction of desensitization and tolerance.

### *Effects on T cells*

Another main goal of allergen-specific immunotherapy is the induction of T cell tolerance and restoration of the $T_h2$- $T_h1$ balance. It is considered that generation of allergen-specific regulatory T cells is a crucial step into T cell tolerance induction together with induction of effector T cell deletion or anergy (17). Some studies have reported that antigen-specific $T_r1$ cells are abundantly found in healthy individuals implying their role in preventing unwanted immune responses to harmless and beneficial environmental antigens (110). Additional factors that are found to be of high importance are IL-10 and TGF-β cytokines which are increasingly produced by allergen-specific $T_{reg}$ cells and are crucial for initiating peripheral T cell tolerance by suppressing allergen-specific effector cell proliferation and skewing cytokine production toward a $T_h1$ phenotype (29). $T_{reg}$ cells have been subdivided in two main subsets: 1) naturally occurring, thymus-derived $Foxp3^+CD4^+CD25^+$ $T_{reg}$ cells (further referred to as $nFoxp3^+$ $T_{regs}$) and 2) induced $Foxp3^+CD4^+CD25^+$ $T_{reg}$ (further referred to as $iT_{regs}$) which are generated peripherally under various tolerogenic conditions. Further, the $iT_{reg}$ cells can be subdivided in TGF-β-secreting ($T_h3$) and IL-10-secreting ($T_r1$) $T_{reg}$ cells which have been shown to play an important role in tolerance to allergens and to be induced during allergen-specific immunotherapy in humans (61, 111). The cytokine profile of $iT_{reg}$ cells appears to be influenced by the surrounding environment, as it has been shown that $iT_{reg}$ cells induced by mucosal allergens (e.g. food allergens or birch pollen) secrete mainly IL-10 and TGF-β, while $iT_{regs}$ induced in the presence of toll-like receptor ligands are more prone to secreting IL-10 and IFN-γ (111). These differences in cytokine production might point at different mechanisms of controlling the allergen-induced immune response by $T_{reg}$ for the different allergen immunotherapies.

Not so many studies have been monitoring the changes in T cells and cytokine production during immunotherapy in food allergy and the few that did, have not contributed consistent results. Due to the low percentage of $T_{reg}$ cells in blood, it is difficult and not always reliable to analyse total $T_{reg}$ numbers in blood. Instead, *in vitro* PBMC stimulation with allergen has been extensively used to determine the capacity of inducing allergen-specific $T_{reg}$ cells. In one peanut OIT study it has been observed that upon stimulation of PBMC with peanut, $Foxp3^+$ $T_{reg}$ cell numbers increased initially, but decreased after 12 months of therapy. Further, increased secretion of IL-10, IL-5 and IFN-γ has been observed (82). On the contrary, during another peanut OIT study the ratio between $Foxp3^{high}/Foxp3^{intermediate}$ $T_{reg}$ cells was increased after 12 months of OIT accompanied by a reduction in IL-5 and IL-13 and transient increase of TGF-β. In this study no changes were detected in IL-10 and IFN-γ levels; however, an initial transient increase in peanut-specific $T_{reg}$ cells was suggested to have influenced the function of peanut-specific T cells later in the treatment (86). Further, cow's milk allergic patients who are able to tolerate heated milk-containing products have revealed a significantly higher percentage of $T_{regs}$ compared to patients who react to heated milk (112).

Interestingly, successful immunotherapy with grass pollen has provided a lot of information on the underlying mechanisms and effects on T cells. Initiation of peripheral T cell tolerance during allergen-specific immunotherapy is contributed to the autocrine action of $T_{reg}$ cells which secrete increasing amounts of anti-inflammatory cytokines, such as IL-10 and TGF-β (111). For example, it has been reported that immunotherapy results in higher IL-10 levels from allergen-stimulated peripheral-T cell cultures (113) as well as in increased mucosal and peripheral IL-10 responses in patients undergoing

grass pollen SIT (114). On the other hand, Jutel *et al.* provided evidence that allergen-induced proliferation of T cells can be suppressed by $T_{reg}$ cells and is mediated by IL-10 and TGF-β (115). In addition, immunomodulatory effects of grass pollen SCIT have revealed changes in peripheral T cell responses and most importantly, a shift towards a $T_h1$ phenotype (116). It is suggested that allergen-specific $T_{reg}$ cells can directly suppress allergen-specific $T_h2$ activation, proliferation and cytokine production (96).

Other ways by which $T_{reg}$ cells manage to control the allergen-specific immune response might involve the anti-inflammatory cytokines IL-10 and TGF-β. In high-dose allergen exposure models, such as bee venom allergy and cat allergy, increased levels of IL-10-producing $T_r1$ cells have been observed (61, 117). IL-10 cytokine appears to be very important for diminishing pro-inflammatory cytokine production, inhibiting IL-5-secretion by $T_h2$ cells, suppressing expression of co-stimulatory molecules by APC and thus downregulating their antigen-presenting capacity. Effects of IL-10 have been proposed in inducing T cell anergy by inhibiting signalling via CD28 and promoting antibody isotype switch from IgE towards IgG4 as previously mentioned (29, 61, 96).

Besides IL-10, TGF-β is also an essential mediator in T cell tolerance. Its main importance is in inducing Foxp3 expression by T cell receptor (TCR)-challenged naïve peripheral $CD4^+CD25^-$ T cells which consequently convert to $CD4^+CD25^+$ $Foxp3^+$ $iT_{reg}$ cells (118). Further, it has been reported to enhance the suppressive capacity and expansion of $T_{reg}$ cells *in vivo* (119). Other important features of TGF-β are the ability to suppress immunoglobulin production, except IgA, and to further inhibit B cell proliferation and differentiation (120, 121). This phenomenon has been demonstrated in allergen-specific immunotherapy with mucosal allergens (e.g. house dust mite and birch pollen) where TGF-β levels are increased and associated with higher amounts of allergen-specific IgA (115).

### *Role of antigen-presenting cells*

The major role of regulatory T cells in oral tolerance and modulation of the allergen-specific immune responses is becoming more and more clear. However, how exactly $T_{reg}$ cells are induced is still under investigation (122). As previously mentioned, when allergen exposure occurs, the first contact with the allergen is with the antigen-presenting cells, being professional APC (e.g. dendritic cells, macrophages, etc.) or non-professional APC (e.g. epithelial cells). In the intestine, both macrophages and dendritic cells are of major importance for oral tolerance and for protective immune responses to pathogens. Similarly, Langerhans cells are the potential counterparts for antigen presentation in the oral mucosa and skin. The tolerogenic features of dendritic cells, previously believed to be intrinsic, appear to result from a complex interplay between the local and cytokine environment. Apparently, dendritic cells in the mucosal immune system play the major role in priming naïve T cells which implies their major role in immunotherapy as they might be involved in the decision which T cell response phenotype to be induced (41). Some DC subsets, such as the ones residing in the intestinal lamina propria and mesenteric lymph nodes, appear to be more prone of inducing $Foxp3^+$ $iT_{reg}$ cells in the presence of exogenous TGF-β compared to splenic DC (50, 123). An example of such tolerogenic DC subsets is the mucosal $CD103^+$ DC in the lamina propria and MLN. Those are found to induce $Foxp3^+$ $iT_{reg}$ cells in the MLN when in the presence not only of TGF-β but also of the vitamin A metabolite, retinoic acid (50). Additional factors present in the gastrointestinal environment, such as the capacity of

intestinal epithelial cells and monocytes to produce IL-10 and TGF-β, may thus contribute to $iT_{reg}$ development and antigen-specific IgA production, with the latter being able to prevent inflammation by mean of immune exclusion (17). Modulatory effect on immunoglobulin isotypes has been demonstrated by skin dendritic cells as well which manage to boost systemic IgM, IgG and mucosal IgA responses after allergen presentation at the regional lymph nodes (101). In animal studies of airway hypersensitivity, it has been demonstrated that pulmonary dendritic cells exposed to respiratory allergen produce IL-10 and stimulate $T_r1$ cells. Additionally, this group reported that this tolerogenic effect of the pulmonary DC was maintained upon an adoptive DC transfer from the allergen-exposed animals to naïve recipients resulting in hyporesponsiveness of the recipients upon challenge with that allergen (124). Similarly, in other animal studies it has also been implied that the pro-tolerogenic capacity of dendritic cells is IL-10 mediated (125).

As far as the oral mucosa and the epidermis are concerned, the involvement of the potent antigen-presenting Langerhans cells is proposed. Their ability to present antigens is emphasized by their expression of FcεR1, MHC class I and II as well as co-stimulatory and co-inhibitory molecules (17, 96). Oral and skin Langerhans cells are suggested to capture antigen by means of allergen-specific IgE bound to the surface FcεR1receptor. The uptake of the antigen stimulates the migratory capacity of these cells which then travel to the local lymph nodes where they present the antigen to T cells and induce the production of anti-inflammatory $T_h1$ and $T_{reg}$ cells (96). It has been shown in *in vitro* assays that oral mucosa Langerhans cells have the ability to produce IL-10 and this ability is additionally enhanced by ligating TLR4 on their surface (126). From this study it appears that TLR4 ligation on Langerhans cells influences T cell responses in co-culture experiments, by diminishing T cell proliferation and skewing the response to a regulatory phenotype. The IL-10, together with TGF-β, secreting capacity of those cells has been observed in human studies with grass pollen-specific immunotherapy. It has been demonstrated that Langerhans cell-produced IL-10 and TGF-β stimulated the development of allergen-specific $T_h3$ subtype $T_{reg}$ cells (127). Other suggestions about the potential effects of Langerhans cells on T cell responses include direct interaction with allergen-specific $T_h2$ leading to suppression of their activity and driving their switch to $T_h1$ or $T_{reg}$ phenotype (96).

Further studies are needed to shed more light to the role of dendritic cells and Langerhans cells in inducing tolerance, so such mechanisms can be utilized in developing safer and more efficient allergen-specific immunotherapy.

## Future improvements for allergen-specific immunotherapy

Even though efficacy has been demonstrated in clinical trials for immunotherapy in food allergy, the frequent occurrence of unwanted and sometimes life-threatening side-effects has prevented this therapeutic approach from being accepted in clinical practice. Interestingly, even in respiratory hypersensitivities where allergen SIT is already available, there is a need for improvement in therapy adjuvants. Systemic reactions and anaphylaxis are first main concern and different novel approaches have been considered to reduce the risk of these possible adverse events (24). One strategy, on which this review will focus, is the use of alternative allergen formulation (allergen peptides) to diminish the allergenicity of the allergen by preventing allergen-mediated IgE cross-linking on effector cells. The other approach to be discussed is encouraged by the observations that not only proteins but also other factors might stimulate tolerance induction. It has been observed that breast-feeding combined with protein exposure has a protective role in preventing sensitization during infancy by inducing tolerance. This tolerogenic effect has been partially attributed to the presence of immunomodulatory factors in human milk, such as non-digestible oligosaccharides (34). It has been suggested that besides positively influencing the gut microbiota, those oligosaccharides can directly exert effects on immune cells (36, 37).

### *Tolerogenic peptides for allergen-specific immunotherapy*

Besides using native allergens in extracts to induce tolerance, it is also possible to use small fractions of the proteins instead. For this purpose small synthetic peptides, comprising T cell epitopes of major allergens, are used as they retain their therapeutic benefit but are less likely to induce side-effects caused by effector cell activation. The main purpose of reducing the size of the allergen is to prevent its ability to cross-link IgE-bound on mast cells and basophils. It has been shown in some studies that the distance between two FcεRI molecules is ranging from 8-24nm, which is approximately 30-100 amino acids (AA). Thus peptides having less than 30 AA, should not be able to cross-link IgE on mast cells. However, it is important to make sure that the allergen fragments do lose their allergenicity, but not their capability of stimulating T cells.

This approach has been more intensively researched in respiratory allergies where synthetic peptides have been tested in a variety of *in vitro*, *in vivo* and clinical studies (128). In murine models of cat and house dust mite allergy, it was found that allergen-specific non-responsiveness or hyporesponsiveness occurs, mediated by reduction in T cell cytokine and antibody production and by down-regulation of T cell responses to the native protein (129, 130). More specific effects have been reported in a bee venom animal study which demonstrated that peptide administration protected from anaphylaxis to the whole protein and reduced specific IgE levels and the $T_h2/T_h1$ ratio (131). In clinical studies, peptide administration by intradermal injection resulted in reduced reactivity to the allergen which might be explained by the skewing of the T cell function toward a regulatory phenotype. In addition, PBMC cytokine profiles revealed reduced production of IL-4, IFN-γ, and IL-13, together with increased secretion of IL-10 (132). Even though clinical studies with peptide immunotherapy have confirmed the positive therapeutic effect seen in *in vivo* models, the frequency of adverse effects seen is still of concern. Thus, more studies are needed to explore sufficiently the potential of this approach.

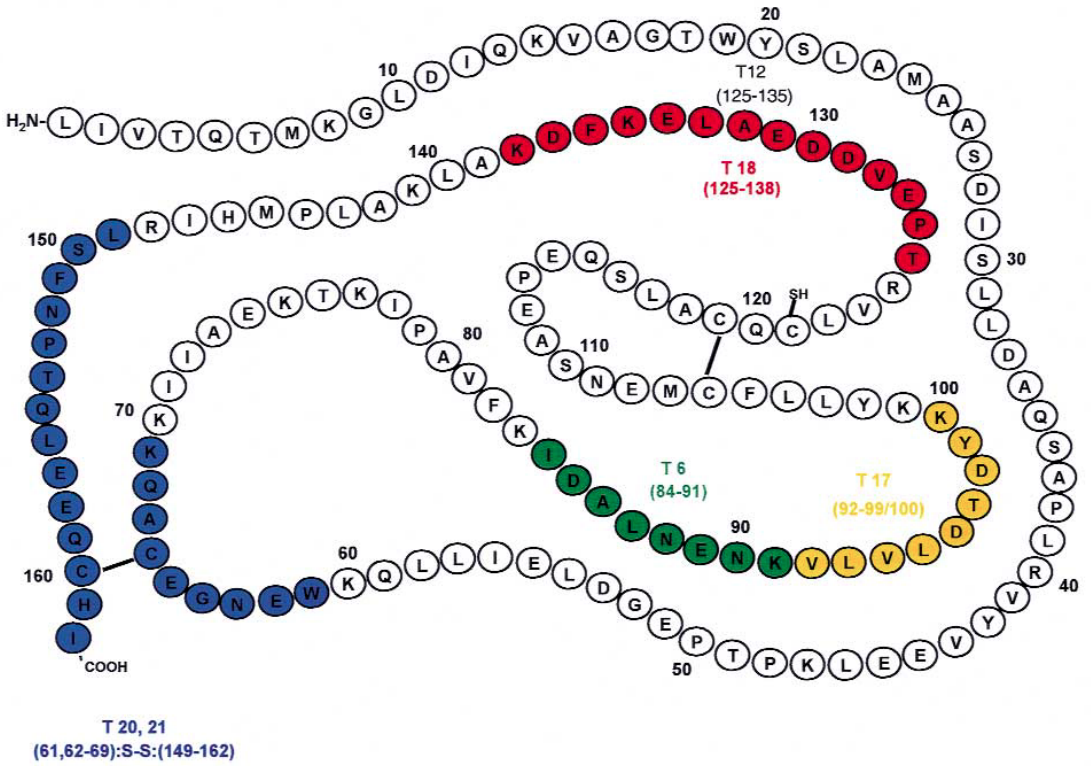


**Figure 2** Sequence of the native bovine β-lactoglobulin showing the location of tryptic peptides which were found to have tolerogenic properties. From Pequet *et al.*

As far as food allergy is concerned, only *in vivo* studies have been performed applying the peptide immunotherapy, however, contributing many promising results. A successful treatment study has been performed in an egg allergy mouse model where multiple T cell epitope peptides from ovalbumin were administered by subcutaneous injection. The therapeutic potential of the peptides has been revealed by the reduced anaphylactic score, lowered allergen-specific IgE levels and increased intestinal expression of TGF-β and Foxp3+. The latter observation suggests the induction of a local intestinal repressive mechanism mediated by regulatory T cells in addition to the skewing towards $T_h1$ effector response observed (133).

In another food allergy *in vivo* study, Pequet *et al.* have demonstrated that tryptic hydrolysis of β-lactoglobulin (β-LG), which is one of the major cow's milk allergens found in the whey fraction of milk, produces peptides which, when given orally, reduce the allergic response to the native β-LG (31). In this preventive study one fraction containing several peptides, mainly T6, T17 and T18 (Figure 2), was shown to be least allergenic (approximately 50 times less allergenic than the total hydrolysate). Further, they have shown that the oral administration of the β-LG peptides results in suppressing β-LG-specific serum IgE and intestinal IgE, accompanied by inhibiting the delayed-type hypersensitivity and proliferative responses; thus the increasing interest has led to the need for further research on the oral administration of these peptides (31).

The use of the peptide strategy for cow's milk allergy has been supported by data concerning the use of milk hypoallergenic formulas in infants with cow's milk allergy who cannot be breast-fed. In addition, it has been found that "at risk" infants who are given cow's milk hypoallergenic formulas (also called hydrolysates) instead of adapted formulas can be prevented from developing allergy. It is hypothesised that this prevention might occur due to the avoidance of the native protein, but might also be due to oral tolerance induction by peptide fractions in the hydrolysates (31). The oral

tolerance induction hypothesis is further supported by *in vivo* studies showing that hydrolysate administration results in increased numbers of $Foxp3^+$ $iT_{reg}$ cells in the MLN which might explain the reduced sensitization and effector response (33). Such hypoallergenic formulas are prepared by enzymatic hydrolysis of the protein of interest, followed by further processing such as heat treatment and ultrafiltration. Further, the hydrolysates have been classified to partial or extensive, depending on the degree of protein hydrolysis. However, it is important to emphasize that extensive hydrolysates have been shown to be ineffective in inducing oral tolerance in a rat model compared to partial hydrolysates (32) suggesting that a high degree of hydrolysis is not preferable as it destroys not only B cell epitopes but T cell epitopes as well.

As most of the studies performed until now are focused on preventing food allergy and sensitization in "at risk" patients, it is important that the exact tolerogenic fragments are elucidated and tested in treatment studies. The interest in the use of tolerogenic peptides for immunotherapy is increased upon the demonstration that tolerance induction by one T cell epitope could confer tolerance to other T cell epitopes of the same protein, suggesting the existence of linked epitope suppression (132). Further, this linked epitope suppression has been shown to be IL-10 dependent as blocking IL-10 abolished the effect (134). Another advantage of this approach is that only few micrograms of purified β-LG tolerogenic peptides will be enough to induce tolerance that will dramatically reduce the dose administered (31).

### *Non-digestible carbohydrates as tolerizing adjuvant*

Dietary interventions have attracted scientific interest due to their potential ability to prevent allergy. This idea has been further supported by observations that breast-feeding can synergize with protein exposure in inducing tolerance (35). One of the abundantly present components in human milk are neutral and acidic non-digestible oligosaccharides (34) which are able of selectively supporting the growth and/or activity of health promoting commensal bacteria in the gut (135), but also directly affect immune cells (36, 37). In this context, a specific mixture (in a ratio 9:1) of non-digestible short-chain Galacto- (scGOS) and long-chain Fructo-oligosaccharides (lcFOS) (Immunofortis®) has been designed to structurally and functionally resemble some of the health promoting properties of the human milk oligosaccharides. When this specific mixture was added to hypoallergenic formulas and used in clinical studies with high-risk infants, it resulted in decreased incidence of atopic dermatitis and allergic reactions by 50% during the first 6 months and the effect was preserved during the following couple of years (37, 136). The latter observation proposes the possibility of immune programming which together with the beneficial total serum antibody profile seen by another group (40) have encouraged the intensive research on the use of scGOS/lcFOS as a tolerizing adjuvant in allergic diseases.

Until nowadays the majority of studies investigating the underlying mechanisms of the non-digestible oligosaccharides immunomodulatory effect in allergic diseases have been in a preventive setting. In an asthma animal model, it has been reported that dietary intervention with scGOS/lcFOS skews the immune response to a $T_h1$ phenotype, by reducing $T_h2$- and activating $T_h1$ pathway (39). This $T_h1$-skewing capacity has been supported by the observation that this mixture of oligosaccharides enhanced the vaccination response in mice, which is primarily $T_h1$-dependent (137). Similarly, in a cow's milk allergy model it was demonstrated that prior exposure to scGOS/lcFOS alone

or in combination with *Bifidobacterium breve* reduces allergic parameters, such as the acute allergic skin response and anaphylactic score, while diminishing mast cell degranulation and stimulating $T_h1$-type serum $IgG_{2a}$ (135). Although allergic symptoms were suppressed in the dietary intervention group, whey specific serum IgE remained unaltered high. However, the dietary intervention was shown to dramatically enhance serum galectin-9 in correlation with the preventive effects. It has been recently found that galectin-9 is a high-affinity IgE-binding lectin which possesses the ability to neutralize IgE antibodies and to induce $T_{reg}$-type immune responses (138, 139). In addition, it has been demonstrated that galectin-9 takes part in the underlying mechanism by which scGOS/lcFOS in combination with *Bifidobacterium* breve reduce allergic symptoms in humans and mice (140). In the latter study galectin-9 in the serum of the protected mice was confirmed to be involved in the suppression of mast cell degranulation (140). To further broaden the understanding of the underlying mechanisms of the effect seen, the same group performed adoptive transfer studies and partial $T_{reg}$ depletion (141) as well as studies with casein allergic mice (142). In their study Schouten *et al.* demonstrated that the oligosaccharide protective effect on allergic symptoms is transferable to a naïve recipient by means of splenocyte adoptive transfer. Further, partial depletion of $CD25^+$ $T_{reg}$ cells revealed that the protective response and the transfer of tolerance are at least partially contributed to allergen-specific $T_{reg}$ cells, which subsequently implies the allergen-specific tolerizing property of the oligosaccharide mixture (141).

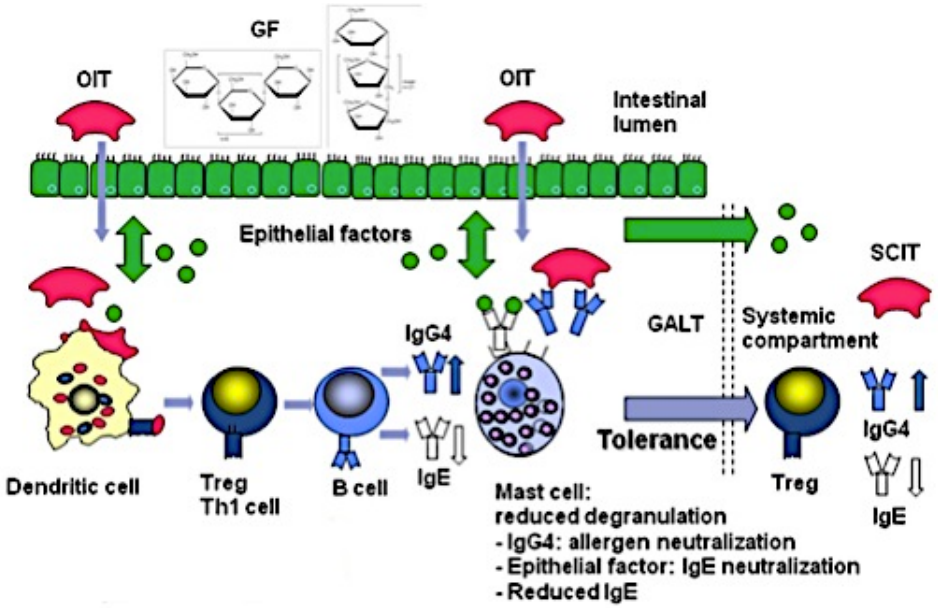


**Figure 3** Proposed mechanisms of action of allergen-specific immunotherapy supplemented with scGOS/lcFOS dietary intervention. Stimulating IEC-derived mediator release by scGOS/lcFOS together with SIT may facilitate induction of tolerogenic $T_h1/T_{reg}$-polarizing response by DC which will further enhance antibody switch from allergen-specific IgE to allergen-specific IgG4. The released IEC factors, such as galectin-9, and the increased numbers of IgG4 will potentially neutralize and block allergen-specific IgE, respectively, and suppress mast cell degranulation. IEC-derived mediators reaching the circulation may have the potential of protecting from allergic symptoms.

Similarly to the *in vivo* studies, *in vitro* studies with scGOS/lcFOS have provided results suggesting the induction of $T_h1$ and $T_{reg}$ cell skewing by the specific mixture of oligosaccharides. Interestingly, this has been observed when the mixture was applied together with a TLR9 ligand (CpG) to an *in vitro* co-culture system containing IEC and activated leukocytes from healthy donors (143). The dependence on IEC presence in the co-culture is not surprising as the initiation and modulation of the immune response in the gut is considered to result from a complex crosstalk between the intestinal epithelial cells and the cells of the innate (APC, mast cells) and adaptive immune systems (B cells and T cells). This complex interplay takes place in the gut associated lymphoid tissue and is determinant for the induction of oral tolerance and maintenance of gut homeostasis. IEC produce soluble factors which might be the modulators of the tolerizing effects of scGOS/lcFOS. Indeed IEC were found to secrete galectin-9 upon apical exposure of scGOS/lcFOS and CpG DNA in the *in vitro* co-culture system. This was shown to contribute to the $T_h1$ and $iT_{reg}$ polarization in the underlying immune cells (de Kivit et al., submitted). Hence, intestinal epithelial cells may provide a source of galectin-9 that is possibly reaching the circulation to prevent mice from developing allergic symptoms in the dietary intervention studies (Figure 3).

The local and systemic availability of IEC-derived mediators, such as galectin-9, would be of great contribution in reducing symptoms, thus improving safety, when co-administered with SIT (mainly OIT or SCIT). However, before any conclusions about the possible beneficial effects of nutritional intervention with scGOS/lcFOS during SCIT are made, more insight in the mechanisms of action are needed, as it is still unknown whether the presence of the allergen in the gut is vital for modulating the allergen-specific response. On the contrary, in the case of OIT, it has been suggested that the persistent side-effects, such as local gastrointestinal ones, are possibly associated with OIT disturbing the intestinal epithelial barrier by activating local effector cells (e.g. mast cells) (107). Thus, scGOS/lcFOS might have not only an immunomodulatory function as a $T_{reg}/T_h1$ polarizing adjuvant, but also a gut barrier protective function by enhancing and regulating the assembly of tight junction proteins (107, 144).

*In vivo* and *in vitro* studies with scGOS/lsFOS have focussed on elucidating the mechanisms underlying the beneficial effects seen with scGOS/lcFOS as nutrition-based adjuvant and their possible implication not only in prevention, but also in therapeutic approaches. Even though most of the studies performed until today are investigating the preventive potential of scGOS/lcFOS, its potential use as a nutritional supplement in novel therapeutic strategies would ideally enhance their efficacy, by supporting the establishment of permanent oral tolerance, and safety, and by minimising the amount of adverse events.

## Conclusion

Increasing interest in the establishment and pathophysiology of food allergy, and the possibilities in managing it actively has encouraged many studies. The intensive research and the amount of data already available indicate the possibility of having a successful treatment option for food allergy in the near future. From all the different approaches being studied, SCIT has a great potential for efficacy, but it is, unfortunately, accompanied by many and severe side-effects which has diminished the enthusiasm of using this approach in food allergy for now. On the other hand, OIT has gained the attention due to its ability to induced desensitization with a relatively good safety profile. However, more research is needed into defining the optimal doses and administration protocol in order to accelerate its approval for implementation in clinical practice. A drawback of this approach is the inability to induce tolerance, which might require more studies and more innovative changes in the approach to achieve better efficacy and ideally permanent tolerance. Other promising immunotherapies make use of the sublingual and epicutaneous route of administration, which appear to improve the safety and the convenience of the therapy. Nevertheless, still many uncertainties about their efficacy in inducing desensitization or even tolerance to food allergens are requiring improvements in both approaches.

Among many strategies for refining therapeutic approaches for food allergy, two have been selected for their therapeutic potential as implied by their efficiency in preventing food allergy development. Both improvement strategies presented aim at enhancing efficacy and safety, but in a different manner. The use of tolerogenic peptides, which have significantly reduced allergenicity, has the potential of replacing allergen extracts and thus diminishing the occurrence of adverse events while enhancing the tolerance induction probability. On the other hand, co-administration of a nutrition-based $T_{reg}/T_h1$-polarizing adjuvant, such as the scGOS/lcFOS specific mixture of non-digestible oligosaccharides, to immunotherapy with allergen extracts or tolerogenic peptides might facilitate the induction of tolerance while protecting from allergic symptoms. Even though immunotherapy for food allergy is still not ready for the clinic, there are many innovative and optimistic studies currently going on which aim at collecting enough evidence for the possible implementation of allergen-specific immunotherapy as an intervention for food allergy management.

## References


1. Sicherer SH, Sampson HA. Food allergy: recent advances in pathophysiology and treatment. Annual review of medicine.2009;60:261-277.
2. Branum AM, Lukacs SL. Food allergy among children in the United States. Pediatrics.2009;124:1549-1555.
3. Gupta R, Sheikh A, Strachan DP, Anderson HR. Time trends in allergic disorders in the UK. Thorax.2007;62:91-96.
4. Mousallem T, Burks AW. Immunology in the Clinic Review Series; focus on allergies: immunotherapy for food allergy. Clinical and experimental immunology.2012;167:26-31.
5. Lee LA, Burks AW. Food allergies: prevalence, molecular characterization, and treatment/prevention strategies. Annual review of nutrition.2006;26:539-565.
6. Vickery BP, Chin S, Burks AW. Pathophysiology of food allergy. Pediatric clinics of North America.2011;58:363-376, ix-x.
7. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. The Journal of allergy and clinical immunology.2001;107:191-193.
8. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. The Journal of allergy and clinical immunology.2007;119:1016-1018.
9. Ross MP, Ferguson M, Street D, Klontz K, Schroeder T, Luccioli S. Analysis of food-allergic and anaphylactic events in the National Electronic Injury Surveillance System. The Journal of allergy and clinical immunology.2008;121:166-171.
10. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics.2003;111:1601-1608.
11. Vickery BP, Scurlock AM, Jones SM, Burks AW. Mechanisms of immune tolerance relevant to food allergy. The Journal of allergy and clinical immunology.2011;127:576-584; quiz 585-576.
12. Sicherer SH, Sampson HA. Food allergy. The Journal of allergy and clinical immunology.2010;125:S116-125.
13. Altschul AS, Scherrer DL, Munoz-Furlong A, Sicherer SH. Manufacturing and labeling issues for commercial products: relevance to food allergy. The Journal of allergy and clinical immunology.2001;108:468.
14. Vierk K, Falci K, Wolyniak C, Klontz KC. Recalls of foods containing undeclared allergens reported to the US Food and Drug Administration, fiscal year 1999. The Journal of allergy and clinical immunology.2002;109:1022-1026.
15. Joshi P, Mofidi S, Sicherer SH. Interpretation of commercial food ingredient labels by parents of food-allergic children. The Journal of allergy and clinical immunology.2002;109:1019-1021.
16. Sicherer SH, Noone SA, Munoz-Furlong A. The impact of childhood food allergy on quality of life. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology.2001;87:461-464.
17. Rachid R, Umetsu DT. Immunological mechanisms for desensitization and tolerance in food allergy. Seminars in immunopathology.2012;34:689-702.
18. Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nature reviews Immunology.2003;3:331-341.
19. Chase MW. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc Soc Exp Biol Med.1946;61:257-259.
20. Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. The Journal of allergy and clinical immunology.2005;115:3-12; quiz 13.
21. Burks AW, Laubach S, Jones SM. Oral tolerance, food allergy, and immunotherapy: implications for future treatment. The Journal of allergy and clinical immunology.2008;121:1344-1350.
22. Ozdemir C. An immunological overview of allergen specific immunotherapy -- subcutaneous and sublingual routes. Therapeutic advances in respiratory disease.2009;3:253-262.
23. Nelson HS. Advances in upper airway diseases and allergen immunotherapy. The Journal of allergy and clinical immunology.2005;115:676-684.
24. Akdis CA. Therapies for allergic inflammation: refining strategies to induce tolerance. Nature medicine.2012;18:736-749.
25. Berin MC, Sicherer S. Food allergy: mechanisms and therapeutics. Current opinion in immunology.2011;23:794-800.

26. Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. The Journal of allergy and clinical immunology.1997;99:744-751.
27. Zuidmeer-Jongejan L, et al. FAST: Towards safe and effective subcutaneous immunotherapy of persistent life-threatening food allergies. Clinical and translational allergy.2012;2:5.
28. Nowak-Wegrzyn A, Sampson HA. Future therapies for food allergies. The Journal of allergy and clinical immunology.2011;127:558-573; quiz 574-555.
29. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. The Journal of allergy and clinical immunology.2011;127:18-27; quiz 28-19.
30. Hoyne GF, Callow MG, Kuo MC, Thomas WR. Inhibition of T-cell responses by feeding peptides containing major and cryptic epitopes: studies with the Der p I allergen. Immunology.1994;83:190-195.
31. Pecquet S, Bovetto L, Maynard F, Fritsche R. Peptides obtained by tryptic hydrolysis of bovine beta-lactoglobulin induce specific oral tolerance in mice. The Journal of allergy and clinical immunology.2000;105:514-521.
32. Fritsche R, Pahud JJ, Pecquet S, Pfeifer A. Induction of systemic immunologic tolerance to beta-lactoglobulin by oral administration of a whey protein hydrolysate. The Journal of allergy and clinical immunology.1997;100:266-273.
33. van Esch BC, et al. Oral tolerance induction by partially hydrolyzed whey protein in mice is associated with enhanced numbers of Foxp3+ regulatory T-cells in the mesenteric lymph nodes. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology.2011;22:820-826.
34. Hanson LA. Session 1: Feeding and infant development breast-feeding and immune function. The Proceedings of the Nutrition Society.2007;66:384-396.
35. Ivarsson A, Hernell O, Stenlund H, Persson LA. Breast-feeding protects against celiac disease. The American journal of clinical nutrition.2002;75:914-921.
36. Agostoni C, et al. Prebiotic oligosaccharides in dietetic products for infants: a commentary by the ESPGHAN Committee on Nutrition. Journal of pediatric gastroenterology and nutrition.2004;39:465-473.
37. Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. The Journal of nutrition.2008;138:1091-1095.
38. Boehm G, Fanaro S, Jelinek J, Stahl B, Marini A. Prebiotic concept for infant nutrition. Acta Paediatr Suppl.2003;91:64-67.
39. Vos AP, et al. Dietary supplementation with specific oligosaccharide mixtures decreases parameters of allergic asthma in mice. International immunopharmacology.2007;7:1582-1587.
40. van Hoffen E, et al. A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induces a beneficial immunoglobulin profile in infants at high risk for allergy. Allergy.2009;64:484-487.
41. van Wijk F, Knippels L. Initiating mechanisms of food allergy: Oral tolerance versus allergic sensitization. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie.2007;61:8-20.
42. Untersmayr E, et al. Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. The Journal of allergy and clinical immunology.2003;112:616-623.
43. Untersmayr E, et al. Anti-ulcer drugs promote IgE formation toward dietary antigens in adult patients. FASEB journal : official publication of the Federation of American Societies for Experimental Biology.2005;19:656-658.
44. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. The Journal of allergy and clinical immunology.2009;124:3-20; quiz 21-22.
45. Westendorf AM, Fleissner D, Hansen W, Buer J. T cells, dendritic cells and epithelial cells in intestinal homeostasis. International Journal of Medical Microbiology.2010;300:11-18.
46. Manicassamy S, et al. Activation of beta-catenin in dendritic cells regulates immunity versus tolerance in the intestine. Science.2010;329:849-853.
47. Iliev ID, et al. Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells. Gut.2009;58:1481-1489.
48. Bilsborough J, Viney JL. Gastrointestinal dendritic cells play a role in immunity, tolerance, and disease. Gastroenterology.2004;127:300-309.

49. Spahn TW, et al. Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. European journal of immunology.2002;32:1109-1113.
50. Coombes JL, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. The Journal of experimental medicine.2007;204:1757-1764.
51. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol.1997;159:1739-1745.
52. Bashir ME, Louie S, Shi HN, Nagler-Anderson C. Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. J Immunol.2004;172:6978-6987.
53. Prescott SL, Bjorksten B. Probiotics for the prevention or treatment of allergic diseases. The Journal of allergy and clinical immunology.2007;120:255-262.
54. Henson M, Burks AW. The future of food allergy therapeutics. Seminars in immunopathology.2012;34:703-714.
55. Navuluri L, Parvataneni S, Hassan H, Birmingham NP, Kelly C, Gangur V. Allergic and anaphylactic response to sesame seeds in mice: identification of Ses i 3 and basic subunit of 11s globulins as allergens. International archives of allergy and immunology.2006;140:270-276.
56. Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy in childhood. The New England journal of medicine.2003;348:977-985.
57. Brown SJ, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. The Journal of allergy and clinical immunology.2011;127:661-667.
58. Lack G. Update on risk factors for food allergy. The Journal of allergy and clinical immunology.2012;129:1187-1197.
59. Redegeld FA, et al. Immunoglobulin-free light chains elicit immediate hypersensitivity-like responses. Nature medicine.2002;8:694-701.
60. Schouten B, et al. Contribution of IgE and immunoglobulin free light chain in the allergic reaction to cow's milk proteins. The Journal of allergy and clinical immunology.2010;125:1308-1314.
61. Fujita H, Soyka MB, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. Clinical and translational allergy.2012;2:2.
62. Nowak-Wegrzyn A, Fiocchi A. Is oral immunotherapy the cure for food allergies? Current opinion in allergy and clinical immunology.2010;10:214-219.
63. Rolinck-Werninghaus C, Staden U, Mehl A, Hamelmann E, Beyer K, Niggemann B. Specific oral tolerance induction with food in children: transient or persistent effect on food allergy? Allergy.2005;60:1320-1322.
64. Larenas-Linnemann D. Certainties and doubts about sublingual and oral immunotherapy in children. Current opinion in allergy and clinical immunology.2009;9:558-567.
65. Dakin R. Remarks on a cutaneous affection, produced by certain poisonous vegetables. Am J Med Sci.1829;4:98-100.
66. Wells H-G. Experiments with isolated proteins, especially those of the hens egg. J Infect Dis.1911;8:147-153.
67. Freeman J. "Rush" Inoculation. The Lancet.1930;215:744-747.
68. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY. Treatment of peanut allergy with rush immunotherapy. The Journal of allergy and clinical immunology.1992;90:256-262.
69. Schofield A. A case of egg poisoning. The Lancet.1908;171:716.
70. Patriarca C, et al. Oral specific hyposensitization in the management of patients allergic to food. Allergologia et immunopathologia.1984;12:275-281.
71. Patriarca G, Schiavino D, Nucera E, Schinco G, Milani A, Gasbarrini GB. Food allergy in children: results of a standardized protocol for oral desensitization. Hepato-gastroenterology.1998;45:52-58.
72. Patriarca G, et al. Oral desensitizing treatment in food allergy: clinical and immunological results. Alimentary pharmacology & therapeutics.2003;17:459-465.
73. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy.2004;59:980-987.
74. Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. Allergy.2007;62:1261-1269.
75. MacDonald TT, Di Sabatino A. The immunologic basis for gastrointestinal food allergy. Current opinion in gastroenterology.2009;25:521-526.

76. Longo G, et al. Specific oral tolerance induction in children with very severe cow's milk-induced reactions. The Journal of allergy and clinical immunology.2008;121:343-347.
77. Skripak JM, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. The Journal of allergy and clinical immunology.2008;122:1154-1160.
78. Pajno GB, et al. Oral immunotherapy for cow's milk allergy with a weekly up-dosing regimen: a randomized single-blind controlled study. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology.2010;105:376-381.
79. Mehl A, Wahn U, Niggemann B. Anaphylactic reactions in children--a questionnaire-based survey in Germany. Allergy.2005;60:1440-1445.
80. Blumchen K, et al. Oral peanut immunotherapy in children with peanut anaphylaxis. The Journal of allergy and clinical immunology.2010;126:83-91 e81.
81. Clark AT, Islam S, King Y, Deighton J, Anagnostou K, Ewan PW. Successful oral tolerance induction in severe peanut allergy. Allergy.2009;64:1218-1220.
82. Jones SM, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. The Journal of allergy and clinical immunology.2009;124:292-300, 300 e291-297.
83. Hofmann AM, et al. Safety of a peanut oral immunotherapy protocol in children with peanut allergy. The Journal of allergy and clinical immunology.2009;124:286-291, 291 e281-286.
84. Varshney P, et al. Adverse reactions during peanut oral immunotherapy home dosing. The Journal of allergy and clinical immunology.2009;124:1351-1352.
85. Varshney P, Jones SM, Pons L. Peanut oral immunotherapy (OIT) induces immunologic changes supporting the development of tolerance. The Journal of allergy and clinical immunology.2010;125:AB59.
86. Varshney P, et al. A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. The Journal of allergy and clinical immunology.2011;127:654-660.
87. Narisety SD, Keet CA. Sublingual vs Oral Immunotherapy for Food Allergy: Identifying the Right Approach. Drugs.2012;72:1977-1989.
88. Mempel M, Rakoski J, Ring J, Ollert M. Severe anaphylaxis to kiwi fruit: Immunologic changes related to successful sublingual allergen immunotherapy. The Journal of allergy and clinical immunology.2003;111:1406-1409.
89. Enrique E, et al. Sublingual immunotherapy for hazelnut food allergy: a randomized, double-blind, placebo-controlled study with a standardized hazelnut extract. The Journal of allergy and clinical immunology.2005;116:1073-1079.
90. Fernandez-Rivas M, et al. Randomized double-blind, placebo-controlled trial of sublingual immunotherapy with a Pru p 3 quantified peach extract. Allergy.2009;64:876-883.
91. de Boissieu D, Dupont C. Sublingual immunotherapy for cow's milk protein allergy: a preliminary report. Allergy.2006;61:1238-1239.
92. Keet CA, et al. The safety and efficacy of sublingual and oral immunotherapy for milk allergy. The Journal of allergy and clinical immunology.2012;129:448-455, 455 e441-445.
93. Kim EH, et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. The Journal of allergy and clinical immunology.2011;127:640-646 e641.
94. Bagnasco M, et al. Absorption and distribution kinetics of the major Parietaria judaica allergen (Par j 1) administered by noninjectable routes in healthy human beings. The Journal of allergy and clinical immunology.1997;100:122-129.
95. Razafindratsita A, et al. Improvement of sublingual immunotherapy efficacy with a mucoadhesive allergen formulation. The Journal of allergy and clinical immunology.2007;120:278-285.
96. Scadding G, Durham S. Mechanisms of sublingual immunotherapy. The Journal of asthma : official journal of the Association for the Care of Asthma.2009;46:322-334.
97. Mondoulet L, Dioszeghy V, Ligouis M, Dhelft V, Dupont C, Benhamou PH. Epicutaneous immunotherapy on intact skin using a new delivery system in a murine model of allergy. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.2010;40:659-667.
98. Mondoulet L, Dioszeghy V, Vanoirbeek JA, Nemery B, Dupont C, Benhamou PH. Epicutaneous immunotherapy using a new epicutaneous delivery system in mice sensitized to peanuts. International archives of allergy and immunology.2011;154:299-309.

99. Dupont C, Kalach N, Soulaines P, Legoue-Morillon S, Piloquet H, Benhamou PH. Cow's milk epicutaneous immunotherapy in children: a pilot trial of safety, acceptability, and impact on allergic reactivity. The Journal of allergy and clinical immunology.2010;125:1165-1167.
100. Otsu K, Fleischer DM. Therapeutics in food allergy: the current state of the art. Current allergy and asthma reports.2012;12:48-54.
101. Mitragotri S. Immunization without needles. Nature reviews Immunology.2005;5:905-916.
102. Sparber F, Tripp CH, Hermann M, Romani N, Stoitzner P. Langerhans cells and dermal dendritic cells capture protein antigens in the skin: possible targets for vaccination through the skin. Immunobiology.2010;215:770-779.
103. Frew AJ. Allergen immunotherapy. The Journal of allergy and clinical immunology.2010;125:S306-313.
104. Wachholz PA, Durham SR. Induction of 'blocking' IgG antibodies during immunotherapy. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.2003;33:1171-1174.
105. Shamji MH, et al. The IgE-facilitated allergen binding (FAB) assay: validation of a novel flow-cytometric based method for the detection of inhibitory antibody responses. Journal of immunological methods.2006;317:71-79.
106. Carballido JM, et al. T cell epitope specificity in human allergic and nonallergic subjects to bee venom phospholipase A2. J Immunol.1993;150:3582-3591.
107. Leonard SA, Martos G, Wang W, Nowak-Wegrzyn A, Berin MC. Oral immunotherapy induces local protective mechanisms in the gastrointestinal mucosa. The Journal of allergy and clinical immunology.2012;129:1579-1587 e1571.
108. Strait RT, Mahler A, Hogan S, Khodoun M, Shibuya A, Finkelman FD. Ingested allergens must be absorbed systemically to induce systemic anaphylaxis. The Journal of allergy and clinical immunology.2011;127:982-989 e981.
109. Woo HY, et al. Mechanism for acute oral desensitization to antibiotics. Allergy.2006;61:954-958.
110. Akdis M, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. The Journal of experimental medicine.2004;199:1567-1575.
111. Jutel M, Akdis CA. Immunological mechanisms of allergen-specific immunotherapy. Allergy.2011;66:725-732.
112. Shreffler WG, Wanich N, Moloney M, Nowak-Wegrzyn A, Sampson HA. Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. The Journal of allergy and clinical immunology.2009;123:43-52 e47.
113. Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. The Journal of allergy and clinical immunology.2003;111:1255-1261.
114. Nouri-Aria KT, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol.2004;172:3252-3259.
115. Jutel M, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. European journal of immunology.2003;33:1205-1214.
116. Ebner C, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.1997;27:1007-1015.
117. Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. The Journal of experimental medicine.2008;205:2887-2898.
118. Chen W, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. The Journal of experimental medicine.2003;198:1875-1886.
119. Huber S, et al. Cutting edge: TGF-beta signaling is required for the in vivo expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. J Immunol.2004;173:6526-6531.
120. Lebman DA, Edmiston JS. The role of TGF-beta in growth, differentiation, and maturation of B lymphocytes. Microbes and infection / Institut Pasteur.1999;1:1297-1304.
121. Borsutzky S, Cazac BB, Roes J, Guzman CA. TGF-beta receptor signaling is critical for mucosal IgA responses. J Immunol.2004;173:3305-3309.
122. Fujimura T, Okamoto Y, Taniguchi M. Therapeutic effects and biomarkers in sublingual immunotherapy: a review. Journal of allergy.2012;2012:381737.

123. Sun CM, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. The Journal of experimental medicine.2007;204:1775-1785.
124. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nature immunology.2001;2:725-731.
125. Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. Immunity.2003;18:605-617.
126. Allam JP, et al. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. The Journal of allergy and clinical immunology.2008;121:368-374 e361.
127. Scadding GW, et al. Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.2010;40:598-606.
128. Larche M. Peptide therapy for allergic diseases: basic mechanisms and new clinical approaches. Pharmacology & therapeutics.2005;108:353-361.
129. Briner TJ, Kuo MC, Keating KM, Rogers BL, Greenstein JL. Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. Proceedings of the National Academy of Sciences of the United States of America.1993;90:7608-7612.
130. Hoyne GF, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR. Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. The Journal of experimental medicine.1993;178:1783-1788.
131. Astori M, von Garnier C, Kettner A, Dufour N, Corradin G, Spertini F. Inducing tolerance by intranasal administration of long peptides in naive and primed CBA/J mice. J Immunol.2000;165:3497-3505.
132. Moldaver D, Larche M. Immunotherapy with peptides. Allergy.2011;66:784-791.
133. Yang M, Yang C, Mine Y. Multiple T cell epitope peptides suppress allergic responses in an egg allergy mouse model by the elicitation of forkhead box transcription factor 3- and transforming growth factor-beta-associated mechanisms. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.2010;40:668-678.
134. Campbell JD, et al. Peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. The Journal of experimental medicine.2009;206:1535-1547.
135. Schouten B, et al. Cow milk allergy symptoms are reduced in mice fed dietary synbiotics during oral sensitization with whey. The Journal of nutrition.2009;139:1398-1403.
136. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. Archives of disease in childhood.2006;91:814-819.
137. Vos AP, et al. Dietary supplementation of neutral and acidic oligosaccharides enhances Th1-dependent vaccination responses in mice. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology.2007;18:304-312.
138. Niki T, et al. Galectin-9 is a high affinity IgE-binding lectin with anti-allergic effect by blocking IgE-antigen complex formation. The Journal of biological chemistry.2009;284:32344-32352.
139. Seki M, et al. Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. Clin Immunol.2008;127:78-88.
140. de Kivit S, et al. Galectin-9 induced by dietary synbiotics is involved in suppression of allergic symptoms in mice and humans. Allergy.2012;67:343-352.
141. Schouten B, et al. Oligosaccharide-induced whey-specific CD25(+) regulatory T-cells are involved in the suppression of cow milk allergy in mice. The Journal of nutrition.2010;140:835-841.
142. Schouten B, et al. A potential role for CD25+ regulatory T-cells in the protection against casein allergy by dietary non-digestible carbohydrates. The British journal of nutrition.2012;107:96-105.
143. de Kivit S, van Hoffen E, Korthagen N, Garssen J, Willemsen LE. Apical TLR ligation of intestinal epithelial cells drives a Th1-polarized regulatory or inflammatory type effector response in vitro. Immunobiology.2011;216:518-527.

144. Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. The Journal of nutrition.2009;139:1619-1625.