Immune basis of food allergic reactions

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Abstract

Food allergies are diseases where the normal tolerance response to oral antigens is altered. Recent advances have begun to uncover mechanisms that mediate sensitization to food allergens and maintenance of this disease. Production of alarmins by epithelial cells triggers a cascade that leads to allergen-specific IgE synthesis. A role of IL-9 in mast cell recruitment and amplification of allergic response has also been identified. In recent years, increasing evidence suggests that sensitization to food allergens can be developed by non-oral routes, in particular the skin, which led to the proposal of the ‘dual exposure hypothesis’. Environmental factors such as diet or microbiota can shape the immune system to promote tolerance or sensitization to food antigens. While the mechanism of primary tolerance to food antigens is quite clear, the one leading to permanent tolerance in already food-allergic individuals through immunotherapy is still under study. Understanding the mechanisms by which oral tolerance is suppressed and sensitization is developed will help to identify new targets to develop combinatory therapies for the treatment of food allergies.

Key words: Food allergy, Skin sensitization. Oral tolerance. Microbiota. Allergen-specific immunotherapy.
Resumen

La alergia alimentaria es una enfermedad en la que la respuesta fisiológica normal de tolerancia a los antígenos orales, se encuentra alterada. Los nuevos avances en la investigación han comenzado a desvelar los mecanismos que median tanto en la sensibilización a los alérgenos alimentarios como en la persistencia la enfermedad. En primer lugar, la producción de alarminas por parte de las células epiteliales desencadena una cascada que conduce a la síntesis de IgE específica frente a los alérgenos alimentarios. También se ha identificado a la IL-9 como un protagonista muy importante en el reclutamiento de mastocitos y la amplificación de la respuesta alérgica. Además en estos últimos años se han completado diversas investigaciones que sugieren que la sensibilización a los alérgenos alimentarios puede desarrollarse por vías no orales, en particular através de la piel, lo cual ha llevado a la propuesta de la denominada "hipótesis de la doble exposición". Por último, factores ambientales como la dieta o la microbiota, así mismo pueden moldear el sistema inmunológico para promover la tolerancia o la sensibilización a los antígenos de los alimentos. Si bien el mecanismo de tolerancia primaria a los antígenos alimentarios es bastante claro, el que conduce a la tolerancia permanente en individuos que ya tienen alergia a los alimentos a través de la inmunoterapia aún está en estudio. Comprender los mecanismos mediante los cuales se suprime la tolerancia oral y se desarrolla la sensitización ayudará a identificar nuevos objetivos para desarrollar terapias combinadas para el tratamiento de alergias alimentarias.

Introduction

Food allergies are the result of an altered response to dietary antigens but the mechanism that leads to this altered response is not yet clear. Alterations in the gastrointestinal epithelium or presence of adjuvant activity such as bacterial toxins have been shown to break normal tolerance response to food antigens and promote food allergy in experimental models. However, in the recent years, increasing evidence suggests that sensitization to food allergens can be developed through non-oral routes, in particular the skin. Here we will review recent advances in the understanding of mechanism of sensitization to food allergens as well as environmental factors that modify immune responses to promote tolerance or food allergy. We will also discuss if it is possible to modify permanently pre-existing allergic responses to induce tolerance to foods.

Reprograming of oral tolerance towards sensitization

The normal response to oral antigens is that of oral tolerance. It is defined as an active suppression of antigen-specific immune responses induced in the gastrointestinal tract. Oral tolerance is initiated by CD103+ dendritic cells (DCs), which capture antigen in the lamina propria and migrate to the mesenteric lymph nodes. In response to antigen presentation by CD103+ DCs, naïve T cells differentiate to regulatory T cells (Tregs), through a mechanism dependent on TGF-β and retinoic acid [1-3]. Retinoic acid is required to induce expression of the gut-homing markers CCR9 and α4β7 by T cell [4]. Antigen-specific Tregs expressing gut homing markers migrate from the lymph nodes to the lamina propria, where they expand in response to IL-10 production by CX3CR1+ macrophages [5]. Oral tolerance to food antigens requires Treg induction, as ablation of Foxp3+ Tregs resulted in loss of oral tolerance [5]. In
addition, mutations in the Foxp3 locus, a transcription factor essential for Treg development, are associated with development of severe food allergy [6].

Factors that increase antigen delivery to tolerogenic CD103+ DCs favor the induction of Tregs, such as formation of goblet cell-associated antigen passages (GAPs) in the intestinal epithelium that delivered antigen exclusively to CD103+CX3CR1- DCs [7]. Also, production of mucin by goblet cells increase frequency of GAPs, increasing antigen delivery [7]. In addition, hyperglycosylated mucin MUC2 imprints CD103+ DCs with a regulatory phenotype by the induction of TGF-β, RALDH and IL-10 expression and suppression of inflammatory cytokines, promoting oral tolerance [8]. Microbial signals, sensed by intestinal macrophages, also regulate the phenotype of CD103+ DCs. In response to these microbial signals, macrophages induce production of GM-CSF by ILC3, which acts on DCs and macrophages and promotes Treg accumulation and intestinal homeostasis [9].

Several studies support the tolerogenic nature of oral exposure. Early consumption of food, such as peanuts, fish or wheat has been related with a reduced incidence of food allergy [10-12]. In a trial to determine the effect of early introduction of peanuts in children with high risk for development of peanut allergy, early consumption was associated with a dramatic decreased in the frequency of allergy to this food, that was maintained over time even with interruption of peanut consumption for 12 months [13]. Additional trials have been performed to broaden the findings to general population [14, 15] and to study the effect of early introduction of other foods such as egg, with different results [16-18]. A meta-analysis of randomized controlled trials concluded that early egg or peanut introduction to the diet was associated with lower risk of developing allergies to these foods with moderate certainty [19].
During development of food allergy, normal oral tolerance response to dietary proteins is altered and there is a deviation of T cell responses towards a Th2 phenotype characterized by IL-4 production. IL-4 production is required for B cell class-switching and synthesis of antigen-specific IgE. Although IL-4 has been usually associated with Th2 responses, IL-4 can also be produced by T follicular helper cells (Tfh). Tfh are required for germinal center development and function, and they induce B cell class switching [20]. A recent paper using a model of peanut allergy by airway exposure shows that Tfh, defined as ST2-CXCR5+, are required IgE antibody production and development of peanut allergy [21]. By contrast, using a model of skin exposure to crude peanut extract, there was not induction of CXCR5+ ICOS+ Tfh in the draining-lymph nodes [22]. Thus, the role of Tfh responses in relation with development of food allergy needs to be further elucidated.

In mouse models, the default response to antigens given by the oral route is immune tolerance, and therefore, to mimic food allergic sensitization in mouse models, it is necessary to break oral tolerance response by the use of adjuvants. These adjuvants promote DC maturation, Th2 skewing, and IgE production.

Cholera toxin (CT) has been widely used to sensitize to antigens through the oral route [23, 24]. Oral administration of CT induces increased migration of CD103+ DCs and upregulation of OX40L, which mediates Th2 skewing of naïve T cells [25, 26]. OX40L upregulation on DCs was found to be dependent on IL-33 production by gastrointestinal epithelial cells in response to CT but independent on IL-25 and TSLP [26]. In addition, eosinophils also can contribute to DC activation and migration, by releasing eosinophil-specific granule protein (EPO) in response to peanut and CT [27]. EPO acts as an adjuvant, inducing maturation of CD103+ DCs and promoting allergic sensitization. Similarly, uric acid, an alarmin that is released after cellular
damage, is also elevated in response to antigen feeding and CT and presents adjuvant activity [28]. Induction of Th2 responses to food antigens in presence of CT is associated with suppression of antigen-specific Tregs in the gastrointestinal tract [29]. Fig. 1 summarizes the different mechanisms implicated in development of sensitization to food allergens. Although the use of CT to sensitize through the oral route has been extensively used, CT is unlikely to be relevant for allergic sensitization in humans. By contrast, the use of staphylococcal enterotoxin B (SEB) as an adjuvant may be more relevant in sensitization to food allergens. SEB is a toxin produced by *Staphylococcus aureus*, a strain that has been found to colonize 90% of patients with atopic dermatitis [30]. Oral exposure to SEB promoted intestinal DC maturation and enhanced expression of TIM-4, which was required to induce Th2 polarization *in vivo* [31]. Mice that were sensitized to OVA in presence of SEB presented reduced levels of TGF-β expression by splenocytes re-stimulated with antigen *in vitro*, suggesting that, similarly to CT, SEB also mediated oral tolerance suppression by impairing Treg induction [32]. Other models to study allergic sensitization through the oral route are those modeling genetic susceptibility. An example is the mouse model carrying a gain-of-function mutation at the 709 position of the IL-4Rα chain (IL4raF709 mouse), that inactivates the immunoreceptor tyrosine-based inhibitory motif (ITIM) [33]. Polymorphisms in the IL-4/IL-13 axis are linked with atopy [34] and, likewise, IL4ra709 mice are prone to develop food allergy by oral sensitization even in the absence of an external adjuvant. Similar to the models using adjuvants, development of food allergy to oral antigen in this model is associated with a defective allergen-specific Treg induction in the gastrointestinal tract [35]. IL-4 production by mast cells in response to oral antigen suppresses Treg induction and promotes a reprogramming of Treg cells into a Th2 pathogenic phenotype [36]. ILC2 can also produce high levels of IL-4, which contribute to Treg
reprogramming [37]. These pathogenic Tregs express IL-4 and GATA-3, while retaining Foxp3 expression, and are defective in suppressing food allergy. Moreover, PBMCs from milk-allergic children have increased IL-4 and IL-13 expression by milk-specific Tregs after re-stimulation with milk compared to PBMCs from healthy controls or from peanut-allergic individuals without milk allergy [36], suggesting that a Th2 reprogramming of Tregs is also present in food-allergic humans. The mouse models of experimental allergy described above suggest that a functional antigen-specific population of Tregs is required to induce oral tolerance to foods, and exogenous factors that alter the normal tolerant environment of the gut or genetic predisposition may lead to impairment of Treg function and Th2 skewing in the gastrointestinal tract. The role of Tregs in development of tolerance to foods in humans is further support by a study which identified that children who have naturally outgrown food allergy present increased number of antigen-responsive Foxp3+ Tregs and IL-10-expressing CD4+ T cells in comparison to children with active food allergy or non-allergic controls [38, 39]. By contrast, no peanut-specific Tregs were found in PBMCs from children with history of peanut allergy who tolerated a cumulative dose of 1g of peanut and healthy controls after stimulation with peanut, suggesting that naturally occurring tolerance may also be mediated by immunologic ignorance or anergy [40]. Induction of IgG antibodies, especially IgG4, has also been associated with development of primary oral tolerance to foods [16, 41]. IgGs can act as blocking antibody, inhibiting the binding of IgE to the allergen [42] and they are able suppress IgE development and anaphylaxis acting through the inhibitory receptor FcγRIIb [43]. In addition, presence of IgG-allergen immune complexes has been associated with development of tolerance ovalbumin (OVA) in offspring from OVA-sensitized mice. These immune complexes were transferred by breastfeeding and detected by
neonatal Fc receptors present in DCs, and they promote the generation of Tregs [44]. Thus, IgG antibodies have a role in reinforcing the regulatory response to food antigens.

**Role of Mast cells and IL-9**

Mast cells are main players in the effector phase of IgE-mediated food allergy. Degranulation of mast cells due to cross-linking of antigen-specific IgE bound to high—affinity FcεRI induces the release of preformed and newly synthesized mediators that are responsible for allergic symptoms. Mast cell subsets are implicated in different manifestations of food allergy in mice. While connective tissue mast cells are associated with systemic anaphylaxis, both connective tissue and mucosal mast cells are involved in gastrointestinal manifestations of food allergy [45]. However, mast cells are not only implicated in the effector phase but also play a key role in sensitization to food allergens. They produce cytokines such as IL-4 and IL-9 that promote Th2 responses and IgE production and suppress Treg responses [35, 46]. IL-9 is a mast cell growth factor that has a pivotal role in promoting intestinal mastocytosis and food-induced anaphylaxis [47, 48]. A population of mast cells producing high amounts of IL-9 and IL-13 was identified in response to repeated intragastric challenge [46]. These IL-9 producing mast cells (MMC9) were induced by Th2 cells and their depletion suppressed the development of intestinal mastocytosis and food allergy, while transfer of MMC9s restored allergic responses. The role of IL-9 in food allergy was further supported by the finding that *il9* gene transcript in the duodenum of food-allergic patients was upregulated in comparison with control subjects [46]. Moreover, T cells from food-allergic patients that were stimulated with the offending allergen presented increased IL-9 expression than those T cells from control subjects, suggesting that IL-9 is a good marker to differentiate between allergic and non-allergic patients [40, 49, 50]. Mast cells can produce IL-9
in response to the alarmin IL-33, which increases IgE-mediated degranulation and cytokine production and it is critical for the induction of food anaphylaxis after oral challenge [46, 51]. Mast cells also can drive expansion of ILC2, by production of IL-33 and IL-4 which further contributes to amplify the severity of IgE-mediated anaphylaxis [37, 52, 53].

**Sensitization to food allergens through non-oral routes**

The oral route was thought to be the main route for sensitization to food allergens. However, in a majority of cases children experience their first allergic reaction to peanuts on their first known ingestion [54], suggesting that sensitization can occur through non-oral routes or by sensitization during pregnancy.

Sensitization to food allergens *in utero* has been studied analyzing allergen-specific IgE levels from cord blood and newborns blood [55]. Nevertheless, the impact of maternal diet on sensitization is not clear. Some studies suggest that maternal consumption of peanuts during pregnancy could be a risk factor for development of peanut sensitization [56], while other studies found no evidence of prenatal sensitization [57] or a protective role of maternal exposure during pregnancy [58].

*De novo* sensitization to food allergens through the airway route has been described for some antigens, such as seeds or eggs, mainly as an occupational hazard where processing of food can release particulates into the air [59]. Inhalation of lupine or sunflower seed flour and egg proteins by workers have been associated with sensitization and adverse reactions after consumption of these foods [60-62]. Although the airway epithelium constitutes a barrier for the passage of inhaled allergens, environmental risk factors such as respiratory infections or cigarette smoke
exposure, as well as genetic factors altering epithelium may contribute to the development of sensitization by inhalation [63].

**Skin sensitization to food allergens**

There are several lines of evidence that support that early cutaneous exposure to food proteins through a disrupted skin barrier promotes allergic sensitization prior to the first ingestion of food, as opposed to the tolerogenic nature of oral exposure. This led to the formulation of the dual exposure hypothesis, which suggests that exposure to food allergens through altered skin promotes sensitization, while early exposure to food allergens through oral route promotes tolerance [64].

There is a strong association between atopic dermatitis (AD) and food sensitization [65]. Eczematous skin has been considered a major risk factor for food allergy development [66, 67] and children with AD environmentally exposed to peanut allergens show an increased risk of peanut cutaneous sensitization [68, 69]. In addition, an impaired skin barrier function at birth has been described as predictive of food allergy at 2 years of age [70]. Moreover, mutations of Flg (encoding filaggrin) and SPINK5 (encoding serine peptidase inhibitor Kazal type 5), both of which are involved in maintenance of skin barrier function, have been linked to food allergy development [71-74] and similar results have been found in mouse models [75]. Expression of homing makers on allergen-responsive T cells also supports the hypothesis that the initial priming site of T cells occurs through the skin. T cells from peanut-allergic patients that expressed cutaneous lymphocyte antigen (CLA), a skin-homing marker, showed an enhanced proliferative capacity to peanut compared with those that expressed α4β7 integrin, an intestinal-
homing marker [76]. Similarly, peanut-specific T cells from peanut-allergic patients expressed the skin-homing marker CCR4, but low levels of β7 [77].

Although topical exposure has been proposed as a main route of sensitization to food allergens, experimental models have demonstrated that skin is not inherently sensitizing, as topical application of food allergens such as milk in the absence of external adjuvants leads to tolerance [78]. In addition to allergen exposure, epicutaneous sensitization to food allergens may require the effect of additional factors, including skin barrier damage [79] and presence of exogenous adjuvants such as toxins produced by microbes colonizing eczematous skin [22]. Some allergens such as peanut present intrinsic adjuvant activity being able to activate dendritic cells [80] and to sensitize epicutaneously without the use of external adjuvants [22, 81]. Taken together, these evidences support the hypothesis that, under conditions of skin barrier dysfunction or inflammation, sensitization to food allergens can be elicited through the skin.

In experimental models of skin sensitization, gastrointestinal symptoms are induced after oral challenge, demonstrating communication between skin and gut [46, 82-84]. However, the exact mechanism by which this communication occurs has not been completely elucidated. Adjuvant activity in the skin induces the production of epithelial innate cytokines such as TSLP and IL-33 which promote Th2 responses. TSLP is upregulated in models that mimic atopic dermatitis, such as those that employ tape stripping or the vitamin D analog MC903 [79, 84]. In models of TSLP-mediated sensitization, basophils are essential for priming of Th2 responses and development of food allergy [83-86]. TSLP can act directly on basophils [87] or through a cascade initiated by DCs on the skin. DCs upregulate OX40L in response to TSLP and induce IL-3 expression by T cells, which recruits basophils to the lymph nodes [85]. Presence of basophils in the lymph nodes are essential for priming CD4+ T cells to express IL-4, suggesting that basophils could be an
early source of IL-4. IL-33 is also upregulated in tape stripping models of epicutaneous sensitization to food allergens [51]. Moreover, in a model of diarrhea induced by intradermal injection of TSLP and antigen, the IL-33 receptor ST2 was required for TSLP-driven gastrointestinal inflammation [88]. In this model, intradermal injection of IL-33 was sufficient to induce gastrointestinal symptoms in a TSLP-independent manner, suggesting that IL-33 could be acting downstream of TSLP.

In the absence of skin damage, TSLP is not upregulated and IL-33 is central for the development of allergic sensitization to peanut [22]. IL-33 is produced by keratinocytes in response to topical peanut and polarizes skin DCs to drive Th2 responses. IL-33 is also implicated in skin sensitization using SEB as an adjuvant, although SEB induces a broader immunological response than peanut, with induction of both Th2 and T follicular helper cells in a mechanism dependent on IL-33 receptor (ST2), IL-1 and IL-6 [22].

Another epithelial-derived cytokine, IL-25, has also been related with induction of Th2 responses. Intestinal ILC2s respond to IL-25 stimulation by producing high levels of IL-5 and IL-13, which amplify Th2 responses [89]. IL-25 is required for the induction of gastrointestinal allergic responses during oral challenge as mice deficient in IL-25 were resistant to food allergy. However, the role of skin-derived IL-25 in the development of skin sensitization to food antigens needs to be elucidated.

Lipids carried by allergens can also act as immunomodulatory molecules to promote sensitization to allergens [90]. They can be presented by CD1 molecules expressed on the surface of antigen-presenting cells to iNKT cells, acting as adjuvants for sensitization through the skin [91]. Nevertheless, the role of iNKT cells in skin sensitization to food allergens has not been determined yet. iNKT cells are in the interface between innate and adaptive immune
responses and can contribute to allergic sensitization by producing Th2 cytokines. iNKT cells from cow’s milk allergic children were producing higher levels of IL-4 and IL-13 that those from non-allergic children in response to stimulation with lipids present in milk, suggesting their contribution to food allergy [92].

Environmental factors affecting food allergy

Role of microbiota and diet

Some studies have associated altered composition of gut microbiota with risk of food allergy development [93, 94]. In mouse models, food allergy has been associated with a distinct microbiota signature than that presented by mice that did not develop food allergy [95-97]. Furthermore, transplantation of microbiome from Il4raF709 mice, which are prone to develop food allergy through the oral route, to germ-free mice transferred susceptibility to food allergy, pointing out the importance of microbiome composition in food allergic sensitization [96]. Some bacterial strains, such as Clostridia, have been associated with protection from food allergy [97-99]. However, in human studies trying to find bacterial signatures associated with food allergy, the results are diverse [100-102]. Thus, it is not clear yet which microbiota strains promote development of food allergy. Although most of the studies have focused on the contribution of gut microbiota, the skin is also colonized and composition of skin microbiota may also play a role in regulating sensitization to food allergens.

The mechanisms by which microbiota can regulate tolerance or susceptibility to food allergens are diverse. One of the mechanisms that has been described is the regulation of intestinal barrier integrity. Clostridia-containing microbiota can enhance production of IL-22 by innate lymphoid cells and CD4+ T cells, resulting in a reduced intestinal barrier permeability to peanut allergens.
In addition, Clostridia and Bacteroides colonization promotes induction of IgA, which can also reduce allergen transport throughout the epithelial barrier [97].

Several studies support the role of commensal microbiota in regulation of tolerance to food allergens by Treg induction [103]. Germ-free and antibiotic-treated mice have shown a lack of Treg generation in the colonic lamina propria, which is restored following colonization [99, 104-106]. Clostridia species and other bacterial strains such as Bacteroides fragilis and Bifidobacterium promote Treg expansion and enhance regulatory tone of the host immune system [97-99, 107, 108]. Microbiota induces a subset of Tregs expressing RORγt+ that suppress Th2 responses [109]. MyD88 signaling in Tregs is essential to sense commensal microbiota and induce mucosal tolerance [106]. Commensal-derived signals also regulate production of IgE antibodies. Germ-free mice and mice treated with antibiotics present increased basal levels of IgE. Presence of microbiota limits serum IgE and circulating basophils by a mechanism dependent on MyD88 expression by B cells [110].

There are multiple studies supporting interaction between diet and commensal microbiota. Dietary habits induce changes in microbiome composition and bacterial-derived metabolites, regulating pro-inflammatory or tolerogenic responses to food proteins [111, 112]. Carbohydrates indigestible for host enzymes such as dietary fiber can be fermented by gut microbiota, exerting a prebiotic effect that stimulates the growth of beneficial strains and inhibits pathogenic bacteria colonization. Supplementation of maternal diet with galacto-oligosaccharides and inulin during pregnancy and breastfeeding has shown an indirect effect on the offspring giving rise to an increased abundance of Lactobacillus and Clostridium leptum, and a decreased proportion of Clostridium cocoides, which promotes immune tolerance in the offspring to wheat-gliadin [113].

In addition, bacterial metabolic products including short chain fatty acids (SCFAs) have been
shown to directly regulate mucosal immune function and intestinal barrier [114]. Butyrate, a SCFA derived from bacterial metabolism of dietary fiber, has revealed to have an important role in the promotion of functional Foxp3+ Tregs [115-117]. Dietary-derived bacterial SCFAs enhance tolerogenic function of CD103+ DCs via metabolism of vitamin A and RA production, which is associated with Treg expansion and tolerance to food allergens [118]. Dietary intervention in infants showed that formula supplemented with *Lactobacillus rhamnosus* GG was able to expand butyrate-producing bacteria in the gut and alleviated cow’s milk allergic symptoms [119].

Besides carbohydrates, degradation of dietary proteins by intestinal microbiota promotes generation of amino acid-derived metabolites with immunomodulatory properties. Tryptophan-derived metabolites generated by bacteria can activate aryl hydrocarbon receptor (AhR) expressed by epithelial and immune cells [120, 121]. Activation of AhR has been related with suppression of allergic sensitization to egg and peanut allergens by generation of tolerogenic CD103+ DCs and CD25+Foxp3+ Treg cells [122, 123]. In addition, most food allergens are partially resistant to host digestive enzymes, and intestinal microbiota may exert a direct effect on their allergenicity. In celiac disease, it has been shown that certain bacterial strains, such as *Rothia* spp. and *Lactobacillus* spp., are able to degrade non-digested gluten peptides, reducing immunogenicity of these compounds [124, 125]. However, in the context of food allergies, microbial ability to modify food proteins needs to be further investigated.

**Other dietary factors**

Dietary factors can promote a tolerogenic gut environment through direct interaction with host immunity [105]. Low vitamin D levels in serum have been associated with increased risk of food
allergy, while normal levels may confer protective effects [126-128]. Similarly, murine models have shown that vitamin D deficiency exacerbates allergic reactions mediated by increased levels of specific-IgE and reduced percentage of Foxp3+ Tregs as well as altered intestinal epithelial barrier [129, 130]. Vitamin D can directly control IgE production by a mechanism dependent on B cell-derived IL-10, although effect of vitamin D on other cell types is also implicated in regulation of IgE levels [131]. Deficiency of dietary vitamin A has also been associated with breaking of oral tolerance through a decreased expression of RALDH in CD103+ DCs [132]. In addition, intake of this vitamin is required for the efficient generation of RORγt+ Treg cells in response to microbiota signals [109]. Intake of n-3 polyunsaturated fatty acids (PUFAs) have been found to reduce OVA-specific IgE and mast cell protease-1 (MCPT-1) levels and prevent cow’s milk sensitization [133, 134]. In agreement with these results, administration of milk formula enriched in n-3 PUFAs showed a reduction of allergy incidence in early childhood [135]. By contrast, increased intake of vegetable oil rich in n-6 PUFAs has been described to promote allergenic response to whey proteins [136]. Other dietary components that have been associated with prevention of sensitization to food allergens are polyphenols, in particular flavonoids [137-139]. Thus, manipulation of dietary components may help in preventing development of food allergies. Fig. 2 illustrates the role of microbiota and diet in sensitization and tolerance to food.

**Induction of therapeutic tolerance**

Allergen-specific immunotherapy is the most promising treatment currently under investigation for inducing tolerance to foods in allergic patients. Different routes of treatment are being investigated, including the oral, skin and sublingual routes, with oral immunotherapy (OIT) the
route most studied. A subset of patients develops sustained unresponsiveness after cessation of therapy [140-145], with increased tolerance development in young children. Biomarkers to predict effectiveness and durability of the treatment are still not well defined.

In general, allergen-specific immunotherapy has been associated with an early increase in allergen-specific IgE followed by a steady decrease, and increased allergen-specific IgGs, in particular IgG4 [146-150]. As stated before, IgGs can block mast cell and basophil activation, leading to a reduction in Th2 responses and suppression of symptoms [42, 43, 151]. However, suppression of basophil responses during OIT is often transient and levels of antigen-specific antibodies and basophil activation do not always correlate with development of long-lasting responses [143, 152-154]. An increased polyclonal expansion and increased somatic mutation of IgG4 antibodies was found in patients receiving peanut OIT [155, 156]. Also, analysis of the BCR repertoire of Ara h 2-specific memory B cells during peanut OIT identified a convergence in unrelated individuals [157] suggesting that development of a protective IgG4 repertoire able to inhibit IgE binding could be more related with successful clinical outcome than levels of antibody.

Results from human trials and mouse models suggest that the mechanism mediating development of permanent during immunotherapy may not be the same as the one underlying primary oral tolerance to foods. Fig. 3 shows the different mechanisms proposed to mediate symptom suppression during allergen-specific immunotherapy to foods. In particular, there is debate about the role of Tregs in suppressing responses during immunotherapy. In some studies, oral immunotherapy has been associated with expansion of Treg population [150]. Syed and colleagues found that there was a correlation between induction of IL-10-expressing antigen-specific Tregs after peanut stimulation and development of sustained tolerance after OIT [154].
By contrast, other studies found that OIT was associated with reduction of Th2 responses mediated by anergy, but there was no evidence of generation of antigen-specific Tregs in patients with sustained tolerance [158, 159].

Mouse studies support the hypothesis that induction of a functional Treg population may be required for the induction of long-term protection, as the lack of sustained tolerance observed after OIT treatment has been associated with an impaired generation of gastrointestinal Tregs [29, 35]. In studies using IL4raF709 mice, Treg suppression during OIT was dependent on the presence of allergen-specific IgE and blockade of IgE signaling during OIT was effective in re-establishing induction of antigen-specific Tregs in allergic mice and desensitization to food allergens [35]. Blockade of IgE during OIT also promoted generation of functional Tregs in peanut-allergic patients, although effect on sustained tolerance after cessation of therapy was not assessed [160]. A role of Tregs in mediating protection during immunotherapy has been also found in a model of epicutaneous immunotherapy (EPIT). EPIT induced a population of LAP+ Tregs with gut homing properties able to suppress mast cell activation and food-induced anaphylaxis even in the presence of high levels of antigen-specific IgE [29] suggesting that direct suppression of effector cells by Tregs is a mechanism involved in therapeutic tolerance.

**Conclusion**

There is growing evidence supporting the key role of non-oral routes, particularly the skin, in sensitization to food allergens. Key events during sensitization to food allergens are the production of epithelial cytokines, such as TSLP, IL-33 and IL-25, as well as IL-9, which is essential for amplification of allergic responses. Early oral introduction of foods aims to decrease the prevalence of food allergy in children through the process of oral tolerance. Whether
permanent tolerance to food allergens can be induced in food-allergic patients in response to immunotherapy remains to be elucidated, although long-term follow up indicates that there is sustained benefit [142]. There is a need for studies with larger cohorts and longer follow-up of patients to correlate immune modifications with clinical outcome.

The use of combinatory therapies such as OIT in the presence of antibodies blocking IgE signaling and/or IL-9 may be an effective strategy for the successful induction of Tregs in the gut of food-allergic patients and potentially increase the proportion of patients reaching sustained unresponsiveness after immunotherapy treatments. In addition, manipulation of environmental factors such as microbiota and diet to promote tolerance to foods may be effective to develop more effective immunotherapy treatments and prevention strategies.

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References


Figure legends

Figure 1. Mechanism of sensitization to food allergens. Food allergens in the presence of adjuvants such as bacterial toxins, epithelial damage or due to the presence of genetic factors, promote the induction of epithelial cytokines in the gut that act as alarmins inducing DC maturation. DCs present antigens to T cells, inducing Th2 responses. Eosinophil-specific granule protein (EPO) produced by eosinophils can also act as alarmin. Th2 cells produce IL-4, which promotes IgE class-switching on B cells and suppression of Tregs. Tfh can also contribute to B cell class switching and IgE production. Th2 cells produce IL-3 and IL-9 that act on mast cells and amplify allergic responses. IL-33 and IL-25 can also act on ILC2 contributing to suppression of Tregs. In the skin, similar mechanisms have been described. A role of basophils in promoting Th2 responses has also been identified in mouse models. Presentation of lipid carried by antigens to iNKT cells can also act as immunomodulator and may contribute to induce sensitization to food antigens.
Figure 2. Role of microbiota and diet in development of sensitization and tolerance to food antigens. Some strains such as Clostridia have been associated with suppression of sensitization and induction of tolerance. They suppress IgE class-switching, promote Treg induction and reduce intestinal barrier permeability through IL-22 production by ILCs and CD4+ T cells. Microbiota colonization can also promote induction of IgA, which can also contribute to reduce allergen transport throughout the epithelial barrier. Microbiota can also promote food allergy, suggesting that some strains can have a pro-allergenic role. Bacterial metabolic products such as SCFAs and dietary components such as vitamin D, vitamin A, flavonoids and n-3 PUFAs promote tolerance to food allergens by enhancing tolerogenic function of CD103+ DCs and Treg generation. Activation of aryl hydrocarbon receptor (AhR) can be induced by tryptophan-derived metabolites generated by bacteria and has also been associated with suppression of allergic sensitization. By contrast, increased intake of n-6 PUFAs is related with promoting allergenic responses.
Figure 3. Impact of allergen-specific immunotherapy on the immune response to foods. Immunotherapy has been associated with induction of Tregs which suppress Th2 responses, and can also result in anergy or deletion of Th2 cells. B cells undergo somatic hypermutation, with induction of a diverse repertoire of antigen-specific IgG4 and reduced antigen-specific IgE. IgG4 antibodies can act as blocking antibodies. In addition, Tregs can directly block mast cell degranulation. These mechanisms contribute to suppression of symptoms during immunotherapy. The relationship between these mechanisms and clinical tolerance is still unknown.