Immune Basis of Allergic Reactions to Food

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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 the disease. Production of alarmins by epithelial cells triggers a cascade that leads to allergen-specific IgE synthesis. IL-9 has also been shown to play a role in mast cell recruitment and amplification of the allergic response. In recent years, increasing evidence suggests that sensitization to food allergens can be developed via nonoral routes, in particular the skin, thus leading to 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, that leading to permanent tolerance in food-allergic individuals through immunotherapy is still under study. Understanding the mechanisms by which oral tolerance is suppressed and sensitization develops will help to identify new targets to develop combined 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 de la enfermedad. 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 el papel de la IL-9 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 a través 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 pueden moldear el sistema inmunológico para promover la tolerancia o la sensibilización a los antígenos alimentarios. 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 sensibilización ayudará a identificar nuevos objetivos para desarrollar terapias combinadas para el tratamiento de alergias alimentarias.

Palabras clave: Alergia alimentaria. Sensibilización cutánea. Tolerancia oral. Microbiota. Inmunoterapia específica con alérgenos.

Introduction

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

Reprogramming of Oral Tolerance Towards Sensitization

The normal response to oral antigens is that of oral tolerance, which is defined as 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 $\alpha4\beta7$ by T cells [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 induction of Tregs, as ablation of $Foxp3^+$ Tregs has been shown to result in loss of oral tolerance [5]. In addition, mutations in the Foxp3 locus, a transcription factor that is 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 deliver antigen exclusively to $CD103^+CXC3CR1^-$ DCs [7]. Production of mucin by goblet cells increases the frequency of GAPs, thus enhancing antigen delivery [7]. In addition, hyperglycosylated mucin MUC2 imprints $CD103^+$ DCs with a regulatory phenotype through the induction of TGF-β, RALDH, IL-10 expression, and suppression of inflammatory cytokines, thus 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 granulocyte-macrophage colony-stimulating factor (GM-CSF) by innate lymphoid cell (ILC) 3, which acts on DCs and macrophages and promotes accumulation of Tregs and intestinal homeostasis [9].

Several studies support the tolerogenic nature of oral exposure. Early consumption of food, such as peanuts, fish and wheat, has been associated with a reduced incidence of food allergy [10-12]. In a trial to determine the effect of early introduction of peanuts in children with a high risk for development of peanut allergy, early consumption was associated with a dramatic decrease in the frequency of allergy to this food, which was maintained over time, even with the interruption of peanut consumption for 12 months [13]. Additional trials performed to extend findings to the general population [14,15] and to study the effect of early introduction of other foods such as egg have yielded different results [16-18]. A meta-analysis of randomized controlled trials concluded that early introduction of egg or peanut to the diet was moderately associated with a lower risk of developing allergies to these foods [19].

During development of food allergy, the normal oral tolerance response to dietary proteins is altered and there is a deviation of T-cell responses towards a T_H2 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 usually been associated with T_H2 responses, IL-4 can also be produced by T follicular helper cells (Tfh). Tfh are required for germinal center development and function and 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 for IgE antibody production and development of peanut allergy [21]. By contrast, a model of skin exposure to crude peanut extract showed that there was no induction of $CXCR5^+$ $ICOS^+$ Tfh in the draining lymph nodes [22]. Thus, the role of Tfh responses in the development of food allergy needs to be further elucidated.

In mouse models, the default response to antigens via the oral route is immune tolerance. Therefore, to mimic food allergic sensitization in mouse models, it is necessary to break the oral tolerance response by the use of adjuvants. These adjuvants promote DC maturation, T_H2 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 T_H2 skewing of naïve T cells [25,26]. Upregulation of OX40L on DCs was found to be dependent on IL-33 production by gastrointestinal epithelial cells in response to CT but independent of IL-25 and thymic stromal lymphopoietin (TSLP) [26]. In addition, eosinophils can also contribute to activation and migration of DCs 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. 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 T_H2 responses to food antigens in the presence of CT is associated with suppression of antigen-specific Tregs in the gastrointestinal tract [29]. Figure 1 summarizes the different mechanisms implicated in the 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

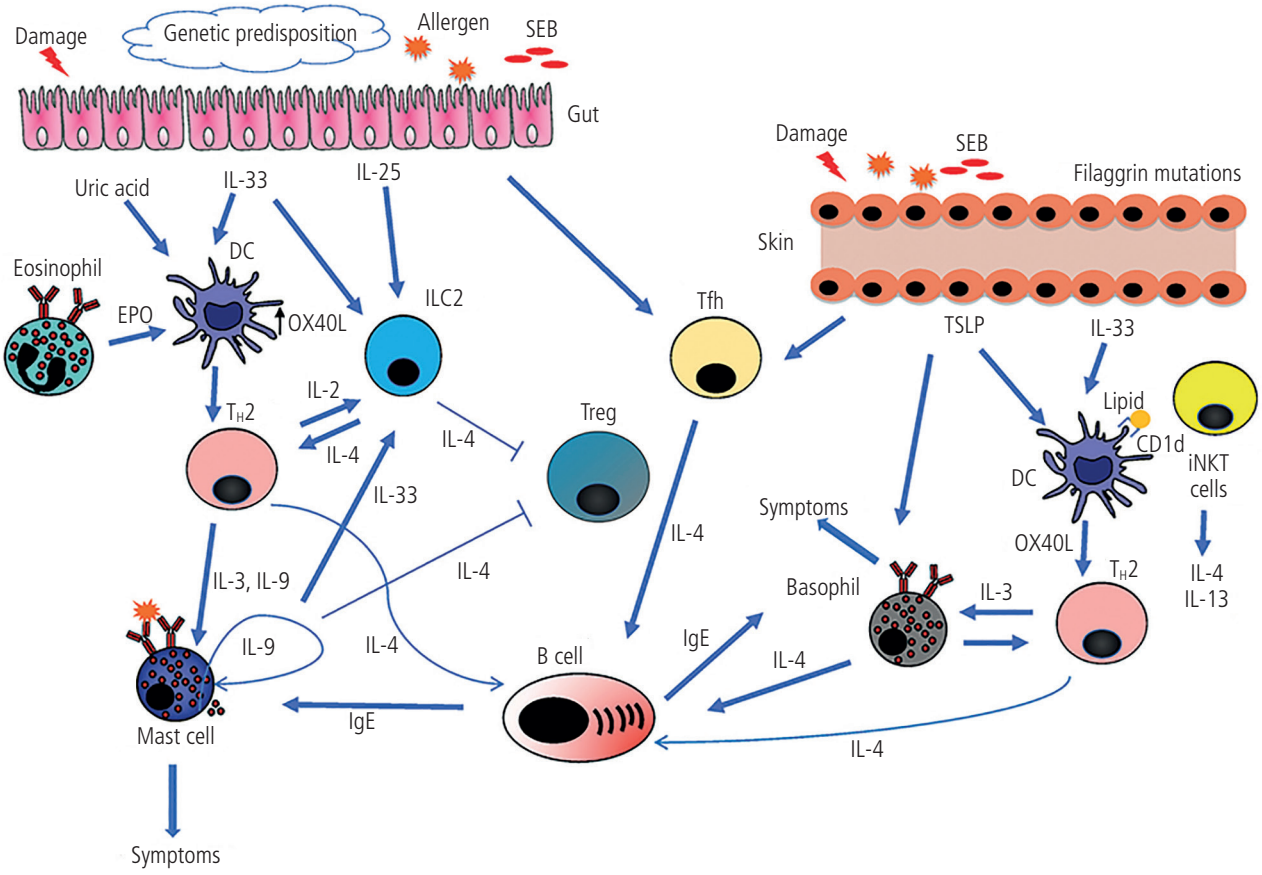

Figure 1. Mechanism of sensitization to food allergens. Food allergens in the presence of adjuvants (eg, bacterial toxins), epithelial damage, and genetic factors promote the induction of epithelial cytokines in the gut that act as alarmins inducing DC maturation. DCs present antigens to T cells, inducing T_H2 responses. EPO produced by eosinophils can also act as alarmin. T_H2 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. T_H2 cells produce IL-3 and IL-9, which act on mast cells and amplify allergic responses. IL-33 and IL-25 can also act on ILC2, thus contributing to suppression of Tregs. Similar mechanisms have been described in the skin. Basophils have also been shown to promote T_H2 responses in mouse models. Presentation of lipid carried by antigens to iNKT cells can also act as an immunomodulator and may play a role in inducing sensitization to food antigens. DC indicates dendritic cell. EPO, eosinophilic granule protein; SEB, staphylococcal enterotoxin B; TSLP, thymic stromal lymphopoietin.

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*, which colonizes 90% of patients with atopic dermatitis [30]. Oral exposure to SEB promoted maturation of intestinal DCs and enhanced expression of TIM-4, which was required to induce T_H2 polarization in vivo [31]. Mice that were sensitized to ovalbumin (OVA) in the presence of SEB presented reduced levels of TGF-β expression by splenocytes restimulated with antigen in vitro, suggesting that, similar to CT, SEB mediated suppression of oral tolerance by impairing induction of Tregs [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 position 709 of the IL-4Rα chain (IL4raF709 mouse), which inactivates the immunoreceptor tyrosine-based inhibitory motif [33]. Polymorphisms in the IL-4/IL-13 axis are associated 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 defective induction of allergen-specific Tregs in the gastrointestinal tract [35]. Production of IL-4 by mast cells in response to oral antigen suppresses induction of Tregs and promotes reprogramming of Treg cells into a T_H2 pathogenic phenotype [36]. ILC2 can also produce high levels of IL-4, which contribute to reprogramming of Tregs [37]. These pathogenic Tregs express IL-4 and GATA-3, while retaining Foxp3 expression, and are defective in suppressing food allergy. Moreover, peripheral blood mononuclear cells (PBMCs) from milk-allergic children have increased IL-4 and IL-13 expression by milk-specific Tregs after re-stimulation with milk compared with PBMCs from healthy controls or from peanut-allergic individuals without milk allergy [36], suggesting that reprogramming of Tregs to T_H2 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 that exogenous factors that alter the normal tolerant environment of the gut and genetic predisposition may lead to impairment of Treg function and T_H2 skewing in the gastrointestinal tract. The role of Tregs in the development of tolerance to foods in humans is further supported by a study showing that children who have naturally outgrown food allergy present higher numbers of antigen-responsive $Foxp3^+$ Tregs and IL-10–expressing $CD4^+$ T cells than children with active food allergy or nonallergic controls [38,39]. By contrast, no peanut-specific Tregs were found in PBMCs from children with a history of peanut allergy who tolerated a cumulative dose of 1 g 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 antibodies, inhibiting the binding of IgE to the allergen [42], and are able suppress development of IgE and anaphylaxis by acting through the inhibitory receptor FcγRIIb [43]. In addition, the presence of IgG-allergen immune complexes has been associated with the development of tolerance to OVA in offspring from OVA-sensitized mice. These immune complexes were transferred by breastfeeding and detected by neonatal Fc receptors present in DCs and promoted 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 various 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 [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, which promote T_H2 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 T_H2 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 expression of IL9 gene transcript in the duodenum of food-allergic patients was upregulated in comparison with controls [46]. Moreover, T cells from food-allergic patients that were stimulated with the offending allergen presented higher IL-9 expression than T cells from controls, suggesting that IL-9 is a good marker for differentiating between allergic and nonallergic 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 is critical for the induction of food anaphylaxis after oral challenge [46,51]. Mast cells can also drive expansion of ILC2, by production of IL-33 and IL-4, which further amplifies the severity of IgE-mediated anaphylaxis [37,52,53].

Sensitization to Food Allergens Through Nonoral Routes

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

Sensitization to food allergens in utero has been studied by analyzing allergen-specific IgE levels from cord blood and newborn blood [55]. Nevertheless, the impact of maternal diet on sensitization remains unclear. 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 airways has been described for some antigens, such as seeds and eggs, mainly as an occupational hazard resulting from release of particulates into the air during food processing [59]. Inhalation of lupine or sunflower seed flour and egg proteins by workers has been associated with sensitization and adverse reactions after consumption of foods containing these ingredients [60-62]. Although the airway epithelium constitutes a barrier for the passage of inhaled allergens, environmental risk factors such as respiratory infections and cigarette smoke exposure, as well as genetic factors altering the epithelium, may contribute to the development of sensitization by inhalation [63].

Skin Sensitization to Food Allergens

There are several lines of evidence that support the hypothesis 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 possibility 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 the 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 development of food allergy [66,67], and children with AD environmentally exposed to peanut allergens show an increased risk of cutaneous sensitization to peanut [68,69]. In addition, impaired skin barrier function at birth has been described as predictive of food allergy at 2 years of age [70]. Moreover, mutations in *Flg* (encoding filaggrin) and *SPINK5* (encoding serine peptidase inhibitor Kazal type 5), both of which are involved in the

maintenance of skin barrier function, have been linked to development of food allergy [71-74]. Similar results have been found in mouse models [75]. Expression of homing markers on allergen-responsive T cells also supports the hypothesis that the initial priming of T cells is through the skin. T cells from peanut-allergic patients expressing cutaneous lymphocyte antigen (CLA), a skin-homing marker, showed an enhanced proliferative capacity to peanut compared with those that expressed $\alpha 4\beta 7$ integrin, a gut- homing marker [76]. Similarly, peanut-specific T cells from peanut-allergic patients expressed the skin-homing marker CCR4, but low levels of $\beta 7$ [77].

Although topical exposure has been proposed as a main route of sensitization to food allergens, experimental models have demonstrated that skin exposure 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 and are able to activate DCs [80] and to sensitize epicutaneously without the use of external adjuvants [22,81]. Taken together, this evidence supports 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 T_H2 responses. TSLP is upregulated in models that mimic atopic dermatitis, such as those that use tape stripping or the vitamin D analog MC903 [79,84]. In models of TSLP-mediated sensitization, basophils are essential for priming of T_H2 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]. The presence of basophils in the lymph nodes is 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 T_H2 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 T_H2 and Tfh cells in a mechanism dependent on IL-33 receptor (ST2), IL-1, and IL-6 [22].

Another epithelial cytokine, IL-25, has also been associated with induction of T_H2 responses. Intestinal ILC2s respond to IL-25 stimulation by producing high levels of IL-5 and IL-13, which amplify T_H2 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 yet been determined. iNKT cells are at the interface between innate and adaptive immune responses and can contribute to allergic sensitization by producing T_H2 cytokines. iNKT cells from cow's milk–allergic children produce higher levels of IL-4 and IL-13 than those from nonallergic children in response to stimulation with the lipids present in milk, thus 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 developing food allergy [93,94]. In mouse models, food allergy has been associated with a microbiota signature that is different from 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, thus highlighting the importance of microbiome composition in allergic sensitization to food [96]. Some bacterial strains, such as Clostridia, have been associated with protection from food allergy [97-99]. However, human studies investigating bacterial signatures associated with food allergy have yielded diverse results [100-102]. Thus, it is not clear yet which microbiota strains promote development of food allergy. Although most 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 reduced intestinal barrier permeability to peanut allergens [97]. In addition, colonization by Clostridia and *Bacteroides* promotes induction of IgA, which can also reduce allergen transport throughout the epithelial barrier [97].

Several studies support the role of commensal microbiota in the 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* species promote Treg expansion and enhance the regulatory tone of the host immune system [97-99, 107, 108]. The gut microbiota induces a subset of Tregs expressing RORγt^{+} that suppress T_H2 responses [109]. MyD88 signaling in Tregs is essential to be able to sense commensal microbiota and to 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. The presence of microbiota limits serum IgE and circulating basophils by a mechanism dependent on MyD88 expression by B cells [110].

Multiple studies support interaction between diet and commensal microbiota. Dietary habits induce changes in microbiome composition and bacteria-derived metabolites, regulating proinflammatory and tolerogenic responses to food proteins [111,112]. Carbohydrates that are indigestible for host enzymes, such as dietary fiber, can be fermented by the gut microbiota, which exerts a prebiotic effect that stimulates the growth of beneficial strains and inhibits colonization by pathogenic bacteria. Supplementation of maternal diet with galacto-oligosaccharides and inulin during pregnancy and breastfeeding has shown an indirect effect on offspring, giving rise to an increased proportion of *Lactobacillus* species and *Clostridium leptum* and decreased proportion of *Clostridium coccoides* and promoting immune tolerance to wheat gliadin in offspring [113]. In addition, bacterial metabolic products including short chain fatty acids (SCFAs) have been shown to directly regulate mucosal immune function and the intestinal

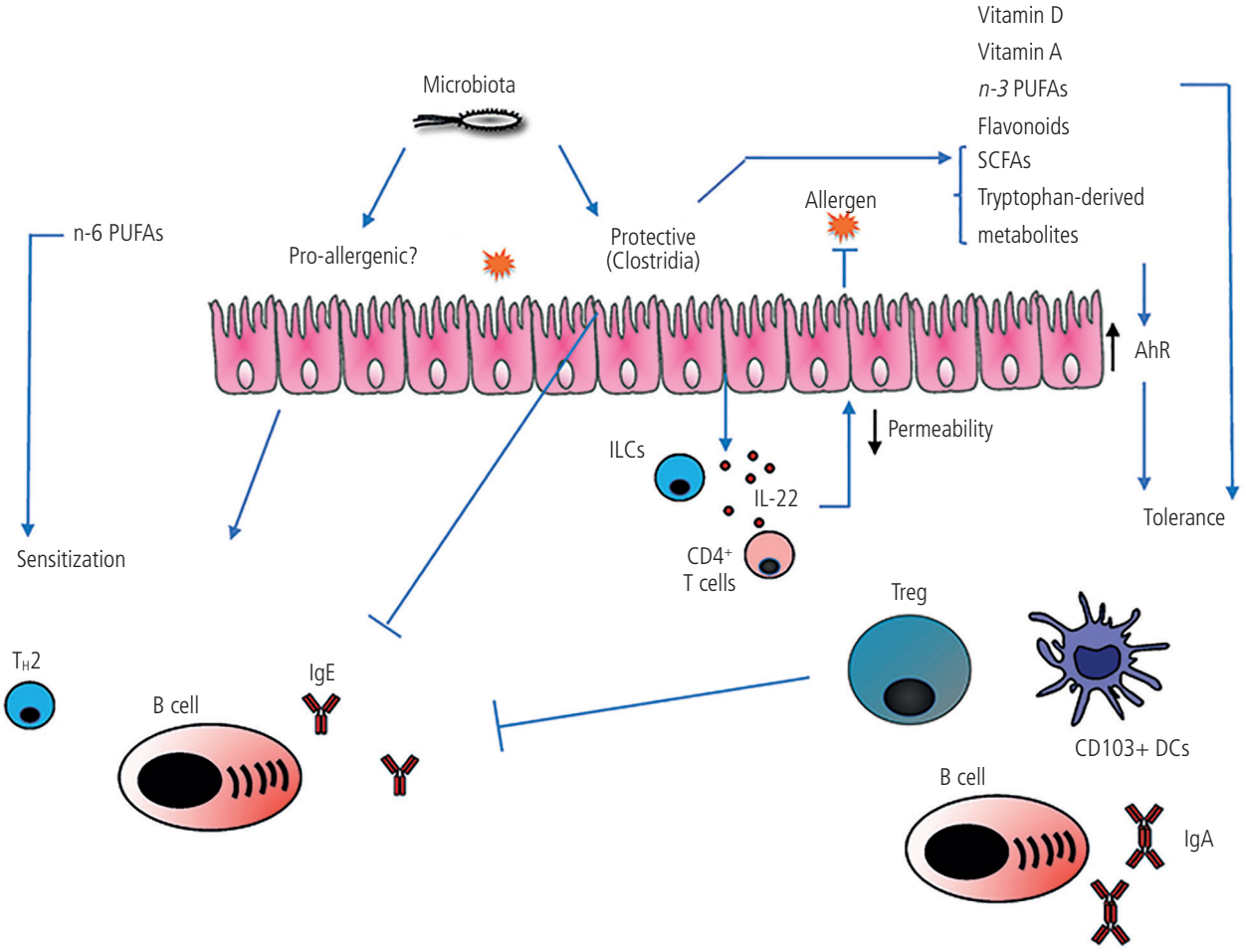

Figure 2. Role of microbiota and diet in the development of sensitization and tolerance to food antigens. Strains such as Clostridia have been associated with suppression of sensitization and induction of tolerance. They suppress IgE class-switching, promote Treg induction, and reduce intestinal barrier permeability through production of IL-22 by ILCs and CD4^{+} T cells. Colonization by microbiota can also promote induction of IgA, which can in turn contribute to reducing 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 the 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. AhR indicates aryl hydrocarbon receptor; DC, dendritic cell; PUFA indicates polyunsaturated fatty acid; SCFA, short-chain fatty acid.

barrier [114]. Butyrate, a SCFA derived from bacterial metabolism of dietary fiber, has proven to have an important role in the promotion of functional Foxp3$^+$ Tregs [115-117]. Dietary-derived bacterial SCFAs enhance the tolerogenic function of CD103$^+$ DCs via metabolism of vitamin A and retinoic acid 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 the symptoms of cow's milk allergy [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 associated 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, certain bacterial strains, such as *Rothia* species and *Lactobacillus* species, are able to degrade nondigested gluten peptides, thus reducing the 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 percentages of Foxp3$^+$ Tregs, as well as an altered intestinal epithelial barrier [129,130]. Vitamin D can directly control IgE production by a mechanism dependent on B cell-derived IL-10, although the effect of vitamin D on other cell types is also implicated in the regulation of IgE levels [131]. Deficiency of dietary vitamin A has also been associated with breaking of oral tolerance through 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) has been found to reduce OVA-specific IgE and mast cell protease-1 (MCPT-1) levels and prevent sensitization to cow's milk [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 include polyphenols, in particular flavonoids [137-139]. Thus, manipulation of dietary components may help to prevent development of food allergies. Figure 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. Various routes of treatment are being investigated, including the oral, cutaneous, and sublingual routes, with oral immunotherapy (OIT) the most widely studied route. A subset of patients develop sustained unresponsiveness after cessation of therapy [140-145], with increased development of tolerance in young children. Biomarkers to predict effectiveness and durability of treatment have not yet been defined.

Figure 3 shows the different mechanisms proposed to mediate suppression of symptoms during allergen-specific immunotherapy to food. In general, allergen-specific immunotherapy has been associated with an early increase in allergen-specific IgE followed by a steady decrease, as well as increased allergen-specific IgG titers, in particular IgG4 [146-150]. As stated above, IgGs can block mast cell and basophil activation, leading to a reduction in T_H2 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]. Increased polyclonal expansion and increased somatic mutation of IgG4 antibodies were found in patients receiving peanut OIT [155,156]. Furthermore, analysis of the BCR repertoire of Ara h 2–specific memory B cells during peanut OIT revealed convergence in unrelated individuals [157], suggesting that development of a protective IgG4 repertoire able to inhibit IgE binding could be more associated with successful clinical outcome than antibody levels.

Results from human trials suggest that the mechanism mediating development of permanent tolerance after immunotherapy may not be the same as the one underlying primary oral tolerance to foods. In particular, there is some debate about the role of Tregs in the development of long-term protection. In some studies, oral immunotherapy has been associated with expansion of the Treg population [150]. Syed et al [154] found a correlation between induction of IL-10-expresing antigen-specific Tregs after peanut stimulation and development of sustained tolerance after OIT. By contrast, other studies found that OIT was associated with reduction of T_H2 responses mediated by anergy, and 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 has been associated with 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 the effect on sustained tolerance after cessation of therapy was not assessed

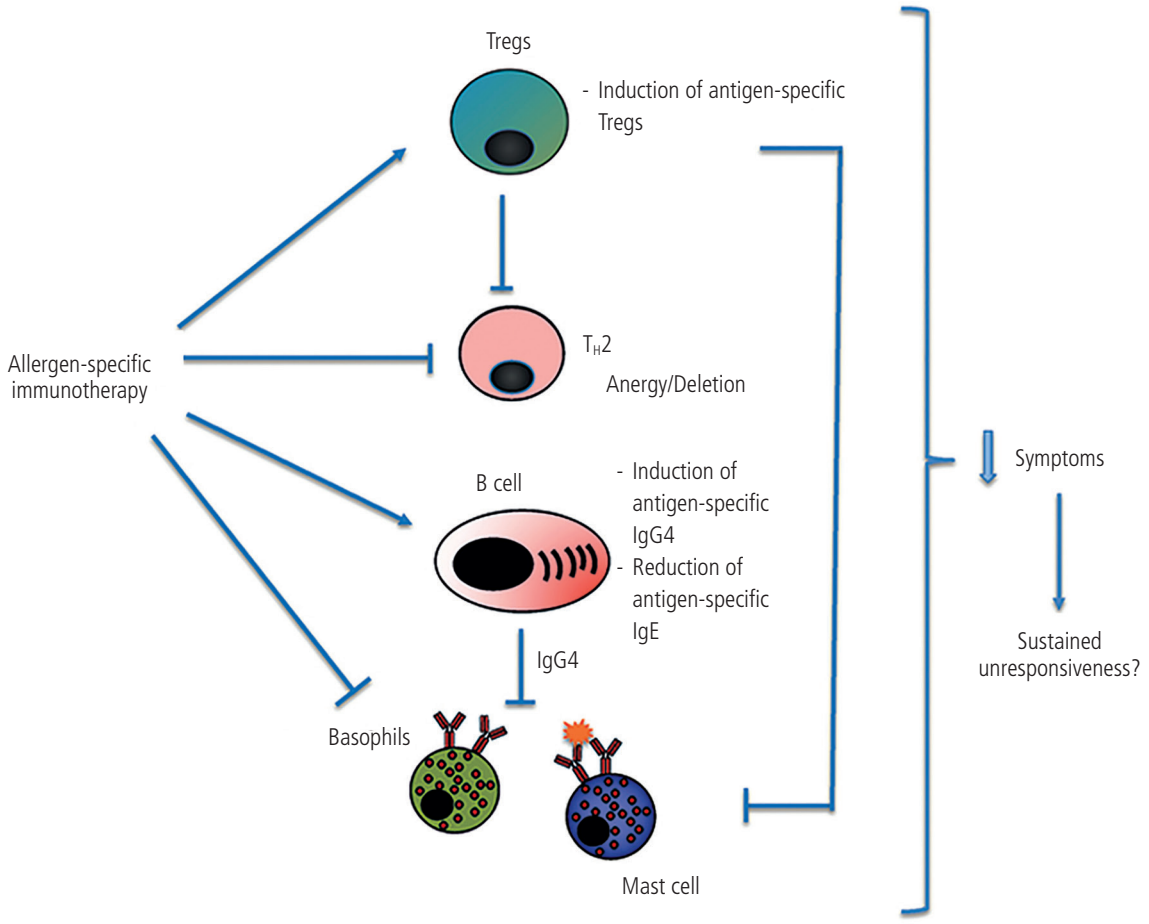

Figure 3. Impact of allergen-specific immunotherapy on the immune response to foods. Immunotherapy has been associated with induction of Tregs, which suppress T_H2 responses and can also result in anergy or deletion of T_H2 cells. B cells undergo somatic hypermutation, with induction of a diverse repertoire 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.

[160]. Tregs were also shown to play a role in mediating protection during immunotherapy in a model of epicutaneous immunotherapy. This approach 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 nonoral routes, particularly the skin, in sensitization to food allergens. A key event during sensitization to food allergens is 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 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]. Studies with larger cohorts and longer follow-up of patients are necessary to correlate immune modifications with clinical outcome.

The use of combined 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. In addition, manipulation of environmental factors such as microbiota and diet to promote tolerance to foods may lead to more effective immunotherapy options and prevention strategies.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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