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REVIEW



Role of dendritic cells in peanut allergy

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ABSTRACT

Introduction: The prevalence of peanut allergy (PA) has increased, affecting approximately 1.1% of children in Western countries. PA causes life-threatening anaphylaxis and frequently persists for life. There are no standardized curative therapies for PA, and avoidance of peanuts remains the main therapeutic option. A better understanding of the pathogenesis of PA is essential to identify new treatment strategies. Intestinal dendritic cells (DCs) are essential in the induction and maintenance of food tolerance because they present dietary allergens to T cells, thereby directing subsequent immune responses.

Areas covered: In this review, we discuss the factors related to the acquisition of oral tolerance to peanut proteins. We focus on intestinal DC-related aspects, including the latest advances in the biology of intestinal DC subtypes, effect of tolerance-inducing factors on DCs, effect of dietary components on oral tolerance, and role of DCs in peanut sensitization.

Expert commentary: Given the increasing prevalence of PA, difficulty of avoiding peanut products, and the potentially serious accidental reactions, the development of novel therapies for PA is needed. The ability of DCs to trigger tolerance or immunity makes them an interesting target for new treatment strategies against PA.

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1. Background

Food allergy (FA) is defined as a reproducible immunologically-mediated response against food [1]. FA affects 6%–8% of children and 2%–4% of adults, representing a growing public health concern worldwide [2]. Foods that generally cause FA include milk, eggs, peanuts, soybeans, tree nuts, wheat, fish, and crustaceans [3,4]; with peanuts as one of the most frequent causes of FA in Western developed countries [2].

Onset of peanut allergy (PA) usually occurs in early childhood and, in 80% of affected individuals, it persists throughout adult life [5]. The prevalence of PA has increased over the last few years, affecting approximately 1.1% (3 million people) of the United States (US) population [4,6]. In line with these data, a recent study by our group showed that PA comprises the most frequent FA among Chilean schoolchildren, with 1.1% prevalence [7]. PA is a common cause of severe allergic reactions, and is responsible for approximately 81% of fatalities due to food anaphylaxis in the US [8].

Currently, there are no standardized curative therapies for PA, and strict avoidance of peanuts remains the main therapeutic option [1]. Given that avoidance of peanuts is difficult and PA is a life-threatening and permanent condition, a low-risk curative therapy for PA that provides long-term immune tolerance to peanuts is required. A better understanding of the pathogenesis of PA is essential to identify new strategies to restore peanut tolerance.

The intestinal immune system (gut-associated lymphoid tissue, GALT) is constantly exposed to various antigens (food, endogenous flora and enteropathogens), and can mount a vigorous immune response when appropriate. The homeostatic balance of tolerance and immunity is modulated by many factors in the intestine and when perturbed, can result in a breach of tolerance and development of inappropriate allergic sensitization to food proteins [9]. Intestinal dendritic cells (DCs) are essential in the induction and maintenance of oral food tolerance by presenting dietary allergens to T cells, and thereby directing subsequent immune responses [10].

Alternatively, sensitization to food allergens may occur via non-oral routes, including the skin. Epidemiological data in children with atopic dermatitis have related the use of moisturizing skin oils containing peanuts with an increased risk of PA development [11]. Additionally, studies have shown that epicutaneous exposure of mice to peanut allergens results in sensitization that is dependent on tape stripping of the skin which leads to upregulation of IL-21 and TSLP [12]. The importance of non-oral sensitization in humans was also suggested by the high rates of PA in high-risk children instructed to avoid dietary peanut in the Learning Early about Peanut (LEAP) study [13]. Nevertheless, although FA is more frequent in children with atopic dermatitis, children with healthy skin also

become sensitized to peanut. These data suggest that the epicutaneous route may have a role in food sensitization, however, it does not account for all the sensitization mechanisms implicated in FA.

In this review, we discuss the factors that lead to the acquisition of oral tolerance to peanuts, focusing on intestinal DCs and the local factors that determine the development of PA, as well as recent evidence of the effect of novel treatment approaches on DCs.

2. Peanut allergens

Each food source contains a mixture of proteins with allergic potential to which the intestinal immune system is exposed, but less than 1% of these proteins induce allergic sensitization in susceptible individuals [14].

Until now, 15 peanut allergens have been identified, termed *Arachis hypogaea* (Ara h) 1 to Ara h 15 (Table 1) [15–18]. Food allergens are classified into families and superfamilies according to their amino acid sequences, structure and function homology [14]. Most peanut allergens are members of the seed storage protein families. The major peanut allergens Ara h 1 and Ara h 2 are members of the cupin and prolamin superfamilies, respectively, and Ara h 2 is the most important member, with a sensitization rate of 42%–100% in PA-affected patients [19,20]. Ara h 8, a protein that is homologous to the major birch pollen allergen *Betula verrucosa* 1 (Bet v 1), is relevant in patients with combined birch pollen and PA, especially in Northern Europe [21]. In contrast, Ara h 9 is a lipid transfer protein, an important allergen in PA patients from the Mediterranean area [21].

3. Immune tolerance to food antigens

Oral tolerance is the absence and/or suppression of antigen-specific immune responses upon dietary intake of a given food. However, the mechanisms involved in the development of oral tolerance have not been fully elucidated [22]. Active gastrointestinal tolerogenic mechanisms appear to be important in preventing FA, in which DCs, regulatory T cells (Tregs), retinoic acid (RA), vitamin D (VD), and microbiota play central role (Figure 1) [23,24]. The different mechanisms involved in the generation of food tolerance are discussed below.

3.1. Role of intestinal immunity in oral tolerance to food

The main route of exposure to food components is through the mucosal surface of the gastrointestinal tract. This mucosa is lined by a monolayer of columnar intestinal epithelial cells joined by tight junctions, which prevent paracellular traffic, thus forming a barrier between the intestinal lumen and connective tissue [25]. The intestinal immune system is the largest and most complex part of the immune system, reaching approximately 100 m² [25]. GALT is composed of organized lymphoid structures and a densely packed population of resident immune cells found in the epithelium and underlying lamina propria (LP). Organized structures of GALT include Peyer's patches (PPs) and isolated lymphoid follicles that are located within the mucosal tissue, as well as mesenteric lymph nodes (MLNs) [23].

After food intake, potentially immunogenic proteins are first subject to denaturation and degradation via digestion in the gut. However, a small percentage of intact proteins escape digestion processes and reach the intestinal lumen, where

Table 1. Classification and clinical implications of peanut allergen components.

Allergen	Protein family	Protein super family	Clinical relevance	Ref.
Ara h 1	Vicilin (7S globulin)	Cupin	Major peanut allergen. Structurally stable molecule associated with severe symptoms.	[15,19]
Ara h 2	Conglutin (2S albumin)	Prolamin	Major peanut allergen. Structurally stable molecule associated with severe symptoms. Most important allergen in patients with PA in North America.	[20,26]
Ara h 3	Glycinin (11S globulin)	Cupin	Structurally stable molecule associated with more severe symptoms.	[18]
Ara h 4	Glycinin (11S globulin)	Cupin	Considerate the same allergen than Ara h 3.	[15,18]
Ara h 5	Profilin		Minor allergen. Related with mild symptoms, mostly oral allergy syndrome. May account for peanut sensitivity in pollen allergic patients.	[18]
Ara h 6	Conglutin (2S albumin)	Prolamin	Exhibits an identity with Ara h 2 of 59%. Heat and digestion stable protein and allergenic potency similar to that of Ara h 2.	[18]
Ara h 7	Conglutin (2S albumin)	Prolamin	42% of identity with Ara h 2. Allergenic properties have not been elucidated.	[15]
Ara h 8	Bet v 1 homologue	Pathogenesis- related proteins	May account for peanut sensitivity in patients with birch pollen allergy.	[15]
Ara h 9	LTP	Prolamin	Major peanut allergen in patients from Mediterranean area.	[26]
Ara h 10 and Ara h 11	Oleosin		Associated with severe allergic symptoms. In-shell roasting increase their allergenicity.	[17]
Ara h 12 and Ara h 13	Defensin	Pathogenesis- related proteins	Possibly associated with severe PA.	[16]
Ara h 14 and Ara h 15	Oleosin		Associated with severe allergic symptoms. In-shell roasting increase their allergenicity.	[17]

Ara h: *Arachis hypogaea*; Bet v 1: *Betula verrucosa* 1; LTP: Lipid transfer protein; ref: reference; PA: peanut allergy.

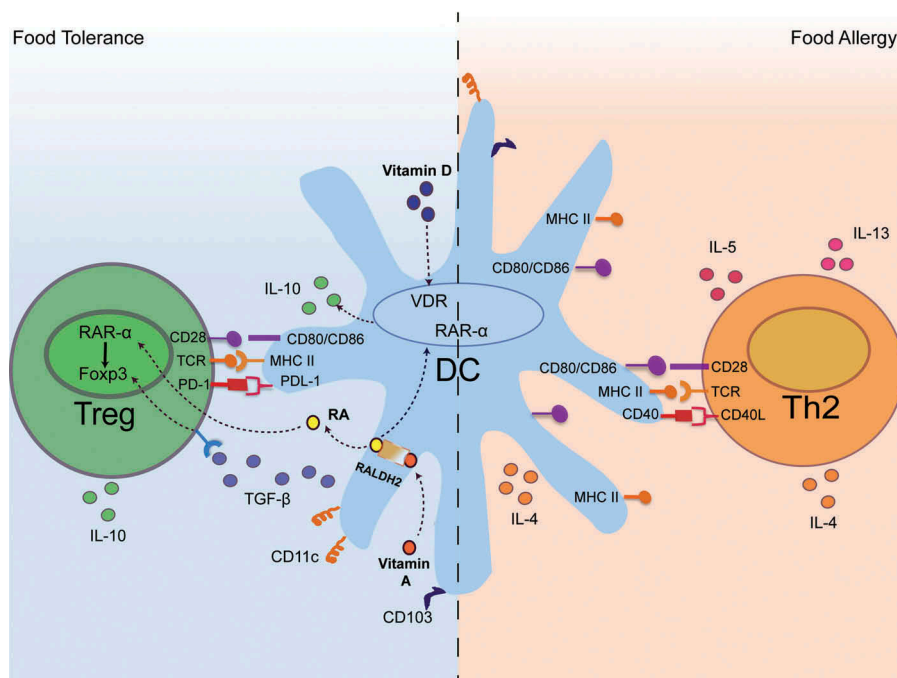


Figure 1. Cellular mechanisms involved in the induction of tolerogenic or activated dendritic cells.

Intestinal CD11c⁺CD103⁺ DCs can capture food allergens with the subsequent development of tolerance or allergy. In the tolerance process, intestinal DCs prime *naïve* CD4⁺ T cells through major histocompatibility complex (MHC) class II (MHC-II)-inducing regulatory T cells (Tregs). Allergen presentation promotes the expression of co-stimulatory molecules, such as CD80, CD86, and programming death ligand-1 (PDL-1), by DCs. These tolerogenic DCs produce the regulatory cytokines transforming growth factor- β (TGF- β) and interleukin-10 (IL-10). Retinaldehyde dehydrogenase-2 (RALDH-2), expressed by intestinal DCs, enables them to convert vitamin A to retinoic acid (RA). RA released to the intestinal mucosal microenvironment act in synergy with TGF- β , promoting the differentiation of *naïve* CD4⁺ T cells into Foxp3⁺ Tregs, which release IL-10. Additionally, RA and Vitamin D stimulate the production of IL-10 through binding to the nuclear receptors of RA (RAR- α) and Vitamin D receptors (VDR). On the other hand, in the development of food allergy, intestinal DCs prime T helper 2 cells (Th2) by up-regulating MHC-II, CD40, CD80 and CD86 expression. Released IL-4 to the intestinal mucosal microenvironment, promotes *naïve* CD4⁺ T-cell differentiation into Th2 cells. These food allergen-specific Th2 cells secrete pro-inflammatory cytokines, such as IL-4, IL-5 and IL-13, generating the typical Th2 response.

they can pass to the intestinal mucosa. In fact, it has been estimated that 2% of intact proteins can pass into the epithelial barrier via several mechanisms, including paracellular diffusion, transcytosis through intestinal epithelial cells, endocytosis by microfold cells (M cells), and sampling by DCs (Figure 2) [26].

DCs are professional antigen-presenting cells that are essential for T-cell activation and play a fundamental role in maintaining peripheral tolerance [27,28]. On the one hand, intestinal DCs can induce tolerance to food antigens and commensal bacteria, but on the other hand, they can also generate a protective immune response against pathogens. In general, DCs are divided into plasmacytoid (pDC) and myeloid DC (mDC) [29]. Classification of DC subtypes is complex due to variations in the expression of cellular surface markers and genes associated to particular subsets, which often results in an overlap of definitions of DC populations between human and mice [30]. The most common classification of intestinal DCs is based on the expression of the surface markers CX3CR1, CD103 and CD11b (Table 2) [31–34]. On one hand, CX3CR1⁺CD103⁺ DCs subtype express high levels of transforming growth factor- β (TGF- β) and retinaldehyde dehydrogenase 2 (RALDH2), an enzyme that catalyzes the synthesis of RA from ingested vitamin A (VA) [35]. These CX3CR1⁺CD103⁺ DCs express CCR7 which allows them to migrate from the LP to the draining MLNs, thereby inducing Foxp3⁺ Tregs [35]. On the other hand, CX3CR1⁺CD103[−] DCs, derived from monocytes, extend their cytoplasmic processes through the epithelium to

sample antigens in the gut lumen [36]. DCs from this subset do not have the ability to migrate to MLNs to activate *naïve* T cells, but are involved in the restimulation of activated effector T cells [36].

Once a food allergen is captured, it can be phagocytosed by DCs or macrophages resident in GALT [25]. The migratory abilities of intestinal CX3CR1⁺CD103⁺ DCs allow them to mobilize from the LP to the draining MLNs, inducing the differentiation of *naïve* CD4⁺ T cells into Tregs [25,35]. In addition, DCs residing within the LP and noninflammatory environment of PPs produce IL-10, which favors the maintenance of intestinal immune tolerance [24]. Subsequently, Tregs activated in the MLNs upregulate expression of the gut-homing receptors integrin $\alpha 4 \beta 7$ and chemokine receptor 9 (CCR9), which induce migration of these activated Tregs through the lymphatic vessels to the intestinal mucosa, interacting at the mucosal surface with the mucosal addressin cell-adhesion molecule 1 (MAD-CAM1) and the chemokine CCL25, respectively [37]. These findings were confirmed in a murine model study that demonstrated that mucosal tolerance requires IL-10 and $\alpha 4 \beta 7$ ⁺CCR9⁺ T cells [38].

The induction of peripheral T-cell tolerance through the generation of allergen-specific Tregs is important in the development of healthy immune responses to allergens [39]. Several sub-types of Tregs related to intestinal immunity have been identified, including CD4⁺ T cells, which secrete TGF- β (Th3); IL-10-secreting type 1 regulatory T cells (Tr1); CD4⁺CD25⁺Foxp3⁺ Tregs; CD8⁺ suppressor T cells; and $\gamma \delta$ T

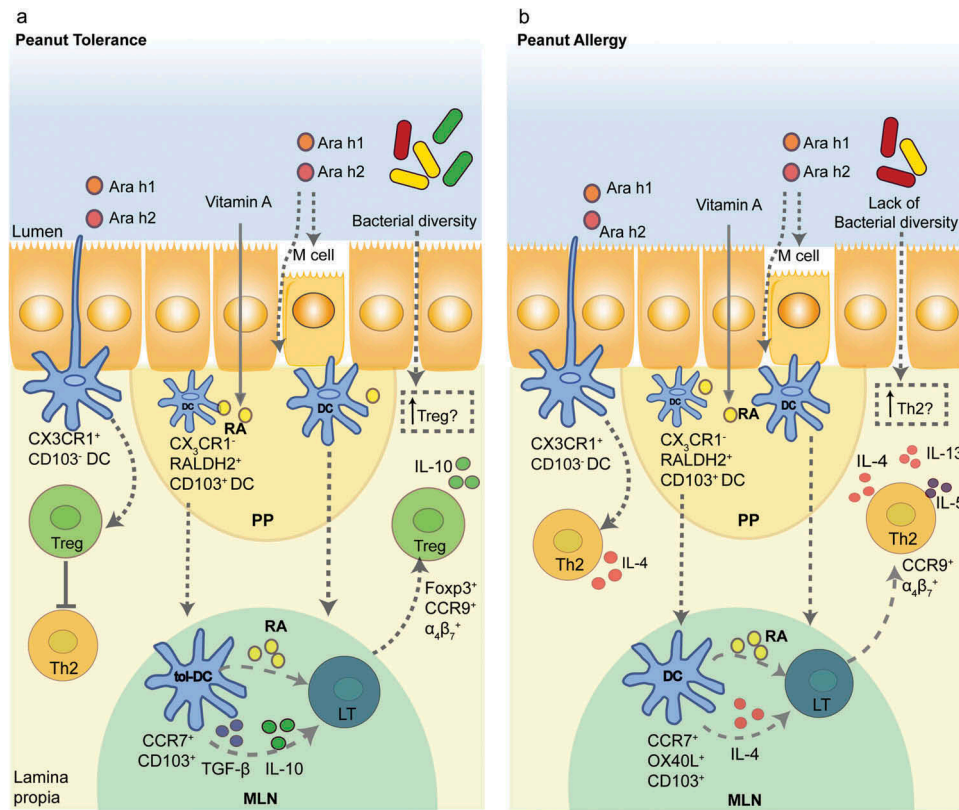


Figure 2. Role of intestinal DCs in peanut tolerance and peanut allergy.

The epithelial cell layer forms the barrier between gut lumen and gut-associated lymphoid tissue (GALT). GALT is composed of organized Peyer's patches (PPs) and mesenteric lymphoid nodes (MLNs). Luminal peanut allergens (e.g. Ara h 1 and/or Ara h 2) can pass the epithelial barrier through paracellular diffusion, transcytosis through intestinal epithelial cells, endocytosis by microfold cells (M cells), or sampling by luminal processes of CX3CR1⁺CD103⁺ DCs. Peanut allergens that reach the intestinal mucosa can be captured by intestinal CX3CR1⁺CD103⁺ DCs. **(a) Tolerogenic mechanisms.** Intestinal CX3CR1⁺CD103⁺ DCs migrate to the MLNs in a CCR7-dependent manner, where they present peanut-allergens derived peptides to naive CD4⁺ T cells. In a non-inflammatory environment, intestinal tolerogenic DCs (tol-DCs) prime naive CD4⁺ T cells into Tregs. Tol-DCs promote Tregs in a mechanism dependent on TGF-β, IL-10, and RA. Foxp3⁺CCR9⁺α4β7⁺ Tregs leave the MLNs in the intestinal mucosa, inducing tolerance to peanut proteins by secreting IL-10. Differentiated Tregs can also be re-stimulated by CX3CR1⁺CD103⁺ DCs in the lamina propria to inhibit the Th2 response. Commensal bacteria may contribute to the induction of Treg differentiation. However, a comprehensive mechanism by which bacteria elicits a Treg response is still unknown. **(b) Mechanisms of peanut allergy (PA).** In the PPs, intestinal CX3CR1⁺CD103⁺ DCs capture the peanut allergen and migrate in a CCR7-dependent manner to the MLNs. Activated CCR7⁺OX40L⁺CD103⁺ DCs present the peanut allergen to naive CD4⁺ T cells inducing differentiation into Th2 cells in an IL-4-dependent mechanism. CCR9⁺α4β7⁺ Th2 cells leave the MLNs to the intestinal mucosa. Peanut-specific Th2 cells promote Th2 immune responses by secreting cytokines such as IL-4, IL-5 and IL-13. Additionally, CX3CR1⁺CD103⁺ DCs can also present peanut-derived proteins to Th2 cells, increasing allergic responses. The lack of diversity of commensal bacteria may increase the Th2 response, however, it is still in doubt whether it is the cause or is a consequence of food allergy.

cells [22]. In a murine model of PA, Smaldini et al. showed that the adoptive transfer of CD4⁺CD25⁺ Foxp3⁺ Tregs was protective against the development of FA and that depletion of CD25⁺ T cells resulted in pronounced disease exacerbation [40]. Additionally, oral feeding with high doses of peanuts induced tolerance by increasing the percentage of CD4⁺CD25⁺Foxp3⁺ in MLNs [41]. However, the role of each individual Treg subtype in oral tolerance remains unclear [26].

3.2. Tolerogenic DC modulation by retinoic acid and vitamin D

Different molecules at physiological and/or pharmacological doses have been shown to induce tolerance in DCs, including most notably RA and VD. VA intake comes from the diet and is responsible for the formation and maintenance of bone, nerve tissue, and retina. VA becomes RA, a molecule with biological activity that exerts numerous effects on the innate and adaptive immune system [42]. RA binds to the RA receptor (RAR), a member of the nuclear receptor family present in most cells of the immune system and other tissues. In the bone marrow, RA induces the differentiation

of DC precursors to pre-mucosal DCs (pre-μDCs) by inducing the expression of α4β7 integrin and CCR9, directing them toward the intestinal LP [43,44]. Pre-μDCs are then differentiated into CD103⁺ DCs, and they acquire the capacity to metabolize VA to RA due to the expression of the RALDH2 enzyme [44]. In MLNs, RA derived from CD103⁺ DCs induces the expression of gut-homing integrin α4β7 and CCR9 by Tregs [45,46]. In addition, the development of Foxp3⁺ Tregs has been attributed to the synergistic effects of RA derived from CD103⁺ DCs and bystander TGF-β [47].

Evidence of Treg induction in oral tolerance was first presented by Belkaid et al. [48]. This study showed that the generation of ovalbumin (OVA)-specific Tregs in MLNs was dependent on TGF-β, RA and CD103⁺ DCs [48]. The effect of RA on Treg differentiation was mediated by RAR-α (RAR-α), a nuclear receptor expressed by DCs and T cells (Figure 1) [42]. Recent studies in murine models of PA show CD103⁺ DC differentiation from bone marrow precursors stimulated with RA *in vitro* [49]. In addition, these DCs are able to inhibit peanut-specific T-cell proliferation by secreting IL-10 and TGF-β, despite the stimulation of DCs with lipopolysaccharide (LPS) [49].

Table 2. Features of human and mouse intestinal DC subtypes.

Subtypes		Features	Ref.
Mouse	Human		
CX3CR1 ⁺ CD103 ⁻ CD11b ⁺	CD103 ⁻ CD11b ⁺	Non-migratory Monocyte-derived LP localization	[31,32]
CX3CR1 ⁻ CD103 ⁺ CD11b ⁺	CX3CR1 ⁻ CD103 ⁺	Migratory capacity (CCR7 ⁺) TGF- β production RALDH2 ⁺ expression Tolerogenic function LP localization	[31]
CD103 ⁺ CD11b ⁺ e-cadherin ⁺	CD103 ⁺ CD11b ⁺ -	PP localization GALT localization	[33] [44]

LP: lamina propria; TGF- β : transforming growth factor β ; RALDH2: retinaldehyde dehydrogenase 2; PP: Peyer's Patches; GALT: Gut-associated lymphoid tissue.

Interestingly, RA has been related to the induction of pathogen-specific pro-inflammatory Th1 and Th17-cell responses. It appears that the regulation of T-cell differentiation by RA depends on the accompanying signals, which dictate whether a response ultimately becomes regulatory or inflammatory [50].

VD, a hormone generally known for regulating calcium and bone metabolism, also exerts immunoregulatory actions and is capable of inducing immune tolerance [51]. VD binds to the VDR, a member of the superfamily of nuclear receptors for steroid hormones, present in most cells of the immune system and other tissues [51]. VD deficiency has been associated with an increased incidence of FA, including PA, in several epidemiological and clinical studies [52]. A pilot study performed in Australia showed that an inadequate VD status at birth may contribute to PA in children, although further studies are required to confirm these results [53].

The VD mechanisms that may contribute to immune tolerance include the induction of tolerogenic DCs (tol-DCs) and Tregs [54]. Human monocyte-derived DCs treated with VD, during LPS-induced maturation, fail to up-regulate the expression of the co-stimulatory molecules CD40 and CD86 [55]. In addition, VD decreases the expression of pro-inflammatory cytokines such as IL-12, and increases the expression of the regulatory cytokine IL-10 via DCs, blocking T-cell activation [55]. VD also stimulates the expression of the inhibitory receptor immunoglobulin-like transcript 3 (ILT3) in DCs, inducing anergy in CD4⁺ T cells [56]. Additionally, tolerogenic VD-treated DCs produce cytokines with suppressor activity and include IL-10 and TGF- β , effectively inducing CD4⁺CD25⁺ Tregs, which can mediate transplantation tolerance [57].

A study in healthy subjects showed that high-dose oral VD supplementation decreased the number of CD103⁺ DCs in the colonic mucosa [58]. Nevertheless, in this study, VD increased the expression of PD-L1 and TGF- β mRNA by T cells, suggesting that DCs maintained their tolerogenic phenotype [58].

3.3. Immunological effect of microbiota on intestinal DCs

In recent years, increasing data has emerged on the role the microbiome plays on the pathogenesis of allergic diseases, including FA. The microbiome consists of complex bacterial, fungal, viral, and protozoan communities that colonize all surfaces of the human body that interact with the outside

world, including the gastrointestinal tract, skin, respiratory tract, and urogenital tract. Bacteria constitute most of the gastrointestinal microbiome, with 10^{11} – 10^{12} bacteria in the colon, and 10^5 – 10^9 bacteria in the small intestine [59]. Alteration of the intestinal microbial population may increase host susceptibility to allergic reaction and other inflammatory immune responses [60].

The colonization of the gut begins in utero and the composition of the intestinal microbiome is constantly changing, a process that is dependent on environmental and dietary factors. Firmicutes and Bacteroidetes are the predominant phyla in the intestine [59]. Because normal gut microbial stimuli are required to develop and maintain the immune system in neonates, a possible role of microbes in achieving oral tolerance to food has been proposed [60]. Mazmanian, et al. showed for the first time in germ-free (GF) mice that commensal bacteria are necessary for the proper development of GALT and to maintain the balance of the Th1/Th2 response [61]. In the same line, Kozakova, et al. proposed that a mixture of three different *Lactobacillus* strains (*L. rhamnosus*, *L. rhamnosus*, and *L. casei*) in GF mice sensitized to the birch pollen allergen *Betula verrucosa* 1 (Bet v 1), ameliorated the Th2 immune response, reducing the Bet v 1-specific IgE serum detection and the production of IL-4 and IL-5 [62]. These findings were related with an improvement in the integrity of the intestinal barrier, which was immature in the non-colonized GF mice [62]. Additionally, microbiota characterized mainly by *Lactobacillaceae* and *Porphyromonadaceae* has been observed in FA-prone mice that have a gain-of-function mutation in the IL-4 receptor α chain [63]. Further allergic sensitization in these mice resulted in the emergence of a FA gut microbiota profile that was significantly different from that of sensitized wild-type mice [63]. Thus, gut dysbiosis could contribute to FA pathogenesis. A similar observation has been reported in 5-year-old children with FA, in whom a high relative abundance of *Clostridium sensu stricto* was associated with an increased risk of FA [64]. Furthermore, another study assessed the richness of the gut microbiota in food-sensitized 3-year-old children, in whom an overrepresentation of *Enterobacteriaceae* was observed [65]. The evidence suggests that the reduction in the diversity of bacteria and/or increased growth of some of these bacteria are implied in the susceptibility to FA during childhood.

Dysbiosis not only affects infants, but also adults. An analysis of the American Gut Project, which evaluated the association of self-reported allergy prevalence with the 16S rRNA sequence from fecal bacteria, showed a decreased richness of the fecal microbiota in allergic adults [66]. Interestingly, the lowest diversity of microbiota was strongly associated with PA compared to shellfish or tree nuts allergy [66]. Furthermore, recent findings in a murine model of PA showed that the dysbiosis induced by an antibiotic therapy could exacerbate the immune response to peanuts allergens plus cholera toxin (CT) [67]. Nonetheless, there is still controversy about whether gut dysbiosis precedes FA or is a consequence of this allergic disease.

4. Breakdown of oral tolerance and sensitization to peanut allergens

FA results from a failure of oral tolerance and is characterized by a Th2 immune response, although the full mechanisms through which FA develops remain unclear [68]. The initial exposure to food allergens occurs at the gastrointestinal mucosa, where specialized epithelial cells transfer food allergens to DCs, which process them and present allergen-derived peptides to *naïve* CD4⁺ T cells via major histocompatibility complex (MHC) class II, inducing either Th2 responses (promoting FA) or alternatively, T regulatory responses (promoting oral tolerance) (Figure 2) [22,35]. In individuals with a genetic predisposition to develop allergic diseases (atopic individuals), activation results in allergen-specific Th2 responses, with concomitant secretion of various cytokines, including IL-4, IL-5, IL-9, and IL-13, which stimulate allergen-specific B cells to produce specific IgE against food allergens, such as peanut proteins [69].

4.1. DCs in the induction of Th2 differentiation and food allergy to peanuts

The importance of DCs in establishing a food-induced allergic response has been shown using adoptive transfer of DCs from mice with cow's milk (CM) allergy into *naïve* recipients, which developed CM-specific IgE and IgG responses in the absence of previous immunization [70]. In addition, it has been shown in murine models, that sensitization to peanut allergens causes a decrease of mucosal tolerogenic CD103⁺ DCs and an increase in Th2 priming CD11c⁺CD11b⁺ DCs in the intestinal compartments [35]. A recent study in children with CM allergy showed that DC-CD4⁺ T-cell co-cultures from CM-allergic children produced spontaneously larger quantities of Th2 cytokines (IL-5 and IL-13) compared with their non-affected siblings [71].

The low capacity to orally sensitize with a food allergen in the absence of an adjuvant in a murine model, suggests that environmental factors that act as adjuvants are important in allergy development [9]. One of the adjuvants that have been used in experimental murine models of FA is CT [72]. The possible underlying mechanism of the coadjuvant effect of CT on DCs, could be the upregulation of the co-stimulatory molecules CD80 and CD86, and the MHC class II molecule [73]. It has also been shown that IL-33, an epithelial Th2-promoting

cytokine, is critical in the development of FA in a CT-induced mouse model of PA [74]. In fact, both CT and IL-33 up-regulated the OX40 ligand (OX40L) on DCs, driving the differentiation of *naïve* CD4⁺ T cells into Th2 cells [74].

There is evidence that some food antigens could have a role in promoting allergic sensitization and type 2-immune sensitization. Specific foods antigens act as adjuvants and stimulate DCs to enhance allergen-specific Th2 differentiation [75]. During PA, the Th2 response is mainly directed by allergens such as Ara h 1 and Ara h 2 [76]. Thus, it was demonstrated that Ara h 1 can bind to the DC-specific C-type lectin receptor, intercellular adhesion molecule non-integrin-gripper 3 (DC-SIGN or CD209), and induce phosphorylation of extracellular signal-regulated kinase (Erk) 1/2 in DCs [76]. Deglycosylated Ara h 1 does not have a Th2-skewing effect, indicating that allergen-bound carbohydrate structures act as a Th2-skewing adjuvant [76]. Furthermore, studies of peanut allergenicity have shown that dry roasting of peanuts can increase sensitization to peanuts in mouse models. Indeed, when the major peanut epitopes Ara h 1 and Ara h 2 were subjected to Maillard reactions (a nonenzymatic chemical reaction between amino acids and reducing sugars occurring at high temperatures), they bound higher levels of IgE from patients with PA and were resistant to digestion [77]. It might be that the allergenicity of various proteins from the environment and food operate in concert to induce allergenic responses.

4.2. DCs and immunotherapy for peanut allergy

Allergen-specific immunotherapy (SIT) has been successfully used to treat allergic asthma, allergic rhinitis and venom hypersensitivity [78-81]. Although the exact underlying mechanisms are still not fully understood, SIT is known to induce allergen-specific Tregs together with an increase in the levels of allergen-specific IgG4 antibodies and reduction of mast cells and basophil activation [82]. Based on the proven effectiveness of SIT for other allergic diseases, different types of SIT strategies for FA treatment have been tested. The first attempts to use SIT for PA utilized subcutaneous SIT but resulted in an unacceptably high rate of systemic reactions and, ultimately, abandonment [83]. Therefore, subsequent studies have focused on alternative routes of delivery of allergenic proteins, including oral-inducing tolerance (OIT), sublingual SIT (SLIT), and more recently, epicutaneous immunotherapy (EPIT) [83,84].

One of the most studied desensitization strategies in PA treatment is OIT, which consists of gradually increasing doses of oral peanut consumption until tolerance is achieved. However, desensitization depends on regular exposure to food because if the dosage is interrupted, the protective effect is lost. An increase in allergen-specific IgG4 antibodies can be observed as early as 8 weeks after starting OIT, followed by a decrease in specific IgE [85]. Peanut OIT induces hyporesponsiveness in basophils, which could be secondary to the decreased surface expression of high-affinity IgE receptors or generation of IgG-blocking antibodies [85]. Regarding the effect of OIT on DCs, Frischmeyer-Guerrero et al. suggested in a pilot study of children with cow's milk allergy, an inhibiting effect of

OIT and SLIT on DCs after treatment [86]. In this study, OIT appeared to enhance the pDC secretion of IFN- α , while SLIT decreased the TLR-induced IL-6 production by mDCs [87]. Increased Tregs have been observed from 9 to 32 months after starting OIT, where successful OIT caused an expansion of allergen-specific CD4⁺ T cells and a shift toward an anergic Th2 phenotype, largely absent in pretreatment allergic subjects and healthy controls [88]. Interestingly, Wisniewski et al. provided evidence that children following peanut OIT have a residual effector IL-4⁺ T cell immune activation, similarly than non-OIT treated children [89]. Suggesting that the Th2 suppression of OIT may not be complete or persistent. In any case, more studies are needed to understand the immunological mechanisms underlying peanut OIT, and if any of these parameters will be useful biomarkers for predicting clinical outcomes.

On the other hand, EPIT utilizes novel antigen delivery through the application of an allergen-containing patch on the epidermis. In a murine model of PA, EPIT induced gut-homing Foxp3⁺ Tregs [90]. A phase II clinical trial of EPIT for PA was recently published, showing that 48% of patients on EPIT containing 250 μ g of peanut protein could tolerate a 10-fold increase in their baseline successfully consumed dose (SCD) [84]. Increases in peanut-specific IgG4 levels and IgG4/IgE ratios were observed in peanut EPIT-treated participants, along with trends toward reduced basophil activation and peanut-specific TH2 cytokines [84]. A recent phase 2 trial with 221 subjects showed results in the same line. Patients with PA (6–55 years old) were randomized to 50, 100, or 250- μ g peanut proteins, or placebo [91]. The primary outcome was passing a 1000 mg peanut protein oral food challenge or achieving ≥ 10 -fold increase in SCD from baseline to week 52. At 12 month there was a significant difference in response rate between the 250- μ g group (50% of response) and the placebo group (25% of response) [91]. Consistent with prior reports, by stratifying by age only in the group of 6–11 years of age was a significant difference between the 250- μ g group versus the placebo group, although this study was not designed to detect an age effect independent of a treatment effect [91].

Alternatively, immunotherapy that specifically targets DCs can be developed. DCs have been evaluated as a therapeutic alternative for inducing tumor-specific immune responses. DCs loaded with allogeneic tumor cell lysates have been tested in several clinical trials for melanoma and other malignancies [92]. This type of immunotherapy has proven to be safe and well tolerated, but still clinical responses are not enough to use as a single treatment, and more research is needed to reach full potential of DCs therapy.

On the other hand, several protocols with different stimuli to induce tolerogenic-DCs *in vitro* have been generated, which include cytokines (IL-10, TGF- β) and immunosuppressive drugs (corticosteroids, VD, RA, rapamycin, rosiglitazone, among others) [93]. Targeting DCs for immune regulation by preloading DCs with specific disease antigens, has been evaluated for tolerance induction in several autoimmune diseases [94]. Studies show beneficial results in animal models of rheumatoid arthritis, multiple sclerosis, and lupus [95]. Clinical trials are now ongoing to evaluate the effectiveness of tol-DCs to treat rheumatoid arthritis, type 1 diabetes,

Crohn disease and multiple sclerosis [96]. Rheumavax is the first study reported for the treatment of rheumatoid arthritis with tolerogenic DCs generated by NF- κ B inhibition, patients received a single intradermal injection of DCs loaded with citrullinated proteins [97]. The treatment was well tolerated and safe, results show a 25% decrease in proinflammatory T cells and a 25% increase in anti-inflammatory Treg cells at one month of treatment, more important, disease activity scores were decreased in some patients. One of the limitations of inducing tolerance with DCs in autoimmune diseases is selecting the proper immunodominant autoantigen [94]. In autoimmune diseases, not all patients display uniform self-antigens, and epitope spreading that has occurred at the time of clinical disease, limits the autoreactive T cell population that can be targeted by DCs [94]. In the setting of FA, antigens are well known and limited, so targeting DCs for immunotherapy in PA may be a promising alternative.

5. Conclusion

Given the increasing prevalence of PA, the difficulty of avoiding peanut products, and the potential serious consequences of accidental reactions, the development of novel therapies for PA is greatly needed. Based on the currently available data from both basic and clinical research that have demonstrated the ability of intestinal DCs to trigger tolerance or immunity, these cells are key interesting targets for new treatment strategies against PA. In addition, the recent discoveries describing the factors that mediate the tolerogenic status of DCs, including RA, VD, and the intestinal microbiome, may provide novel candidate molecules for the deliberate modulation of DC activity.

6. Five year in review

Due to the pivotal role of DCs in FA and knowledge of the different factors that can modulate their pro-tolerogenic state, DCs promise to be an ideal target to develop new therapeutic strategies against PA. Overall, over the next 5 years, additional scientific information will provide important insights into the mechanistic roles of the different subsets of human intestinal DCs in the development of FA, and especially in PA due to its high prevalence.

The most studied desensitization strategies include OIT, SLIT, and recently, EPIT. Over the next years, further understanding of how SIT affects DCs and what strategy is more effective in inducing a pro-tolerogenic state. In addition, given the fact that DCs are probably key at the onset of allergic sensitization and subsequent FA, additional strategies to curb the FA pandemic should focus on inducing a pro-tolerogenic state of DCs in children before FA develops. It is possible that simple clinical interventions such as VD supplementation may have an important effect on DC-mediated tolerance at early ages. Clinical trials of VD supplementation during pregnancy and infancy may provide clues in this regard. Last, the interactions between gut immunity and that of other epithelium, particularly skin, and the effect of these interactions on DCs and induction of tolerance shall

shed light on the precise roles of oral and skin sensitization to peanut and other food allergens.

Future progress in our understanding of oral tolerance in human subjects will advance considerably, allowing more comprehensive analyses of the immune responses in the gut that contribute to sensitization, desensitization, and tolerance. Additionally, new research on the factors that mediate the dialog among intestinal microbes and DCs will provide new candidate molecules for the deliberate modulation of intestinal DC activity.

All these data will provide new lines to explore novel strategies for the treatment of PA, possibly using DCs as a target and the different factors that modulate them as tool to direct the immune response toward oral tolerance.

7. Expert commentary

The prevalence of PA has increased over the last few years, especially in Western developed countries [2,6]. Despite the significant impact of PA on patients and health care systems, there are currently no standardized treatments [1]. Understanding of the immunological mechanisms that lead to the development of FA or oral tolerance is essential to develop new effective treatments for this disease.

The intestinal immune system is constantly exposed to a variety of dietary antigens and commensal microorganisms to which it does not respond. This mechanism, named oral tolerance, is now understood to consist of a complex of factors that lead to the generation of immunosuppressive specific responses. Intestinal DCs are essential in the mechanisms of oral tolerance and FA by presenting dietary allergens to T cells, thereby directing subsequent immune responses [10]. In fact, in a murine model of CM allergy, adoptive transfer of DCs from mice with CM allergy into control *naïve* recipients induces CM-specific IgE and IgG responses in the absence of previous sensitization, demonstrating the essential role of DCs in FA [70]. However, the underlying mechanisms of human DCs are still not well understood.

New data have emerged that provide more specific information on how mucosal DCs have a special tendency to facilitate the development of Tregs that then mediate oral tolerance. For example, the expression of RALDH2 by intestinal CD103⁺ DCs allows them to synthesize RA from the ingested VA [44,58]. Additionally, CD103⁺ DCs can migrate to MLNs, where they can present food antigens to *naïve* T cells. The secretion of RA and TGF- β by CD103⁺ DCs induces the differentiation of CD25⁺Foxp3⁺ Tregs, which express gut-homing signals, such as integrin $\alpha\beta 7$ and CCR9 [45-47]. Interestingly, it was shown that the role of RA in *naïve* CD4⁺ T cells goes beyond the induction of Tregs because pathogen-specific proinflammatory T-cell responses also depend on RA signaling a [50].

Another demonstrated tolerogenic factor that could modulate intestinal DCs is VD, the deficiency of which has been associated with various allergic diseases [98]. Epidemiological studies have suggested that an insufficiency of VD may contribute to the failure of oral tolerance and subsequent FA in infants [52]. There are different mechanisms by which VD can contribute to immune tolerance responses, including induction

of tolerogenic monocyte-derived DCs [55], induction of Tregs [55,56], and induction of anergy in CD4⁺ T cells [56]. However, although it is now clear that VD has wide-ranging effects on the immune system, how it modulates intestinal DCs in terms of FA remains unclear.

To date, there is increasing evidence showing that alterations affecting gut microbiome that promote underlying dysbiosis, could raises the host's susceptibility to allergic and inflammatory responses [60]. For example, it has been shown that GF-mice are more susceptible than wild-type mice to allergic sensitization to CM [99]. Furthermore, recent results from murine models of PA have shown that certain bacteria, most notably those from the Clostridia class, can promote the development of tolerance in the gut. In fact, the colonization of GF/mice with Clostridia-enriched microbiota promotes IgA production and Foxp3⁺ Tregs in the colon, preventing the development of FA [67]. However, the underlying mechanisms regarding how the microbiota modulate the intestinal immune response are not clear, and more data from human studies are needed.

On the other hand, there is some evidence that indicates that food allergens play a role in promoting allergic sensitization, probably by acting as adjuvants in DCs [75]. It has been demonstrated that Ara h 1 can bind to DC209 on DCs and activate them into a Th2 immune effector phenotype [100]. It was suggested that the allergen-bound carbohydrate structure of Ara h 1 could be responsible for this binding, because deglycosylated Ara h 1 did not have a Th2-skewing effect [76]. In addition, it was shown that thermal processing, such as the Maillard reaction, may play an important role in enhancing the allergenic properties of peanut allergens (Ara h 1 and Ara h 2), favoring peanut sensitization [77]. These results suggest that food antigen characteristics can modulate immune responses and induce allergenic responses.

As described above, DCs play an important role in the induction of allergen-specific Treg cells, a key event in SIT. Regarding SIT for FA treatment, the efficacy of OIT and EPIT could be related to their immunoregulatory effects on DCs. However, the underlying mechanisms have not yet been elucidated. Specific-food OIT has superior efficacy but causes more adverse effects than EPIT [85]. Nevertheless, none of these immunotherapeutic approaches have been proven to uniformly cure FA.

Finally, *in vitro*-generated tol-DCs could potentially be used to induce antigen-specific tolerance in various applications, such as autoimmunity, allergy and other hypersensitivity diseases. Several protocols with different stimuli to induce tol-DCs *in vitro* have been developed and include cytokines (IL-10, TGF- β) and immunosuppressive drugs (corticosteroids, VD, RA, rapamycin, rosiglitazone, among others) [93]. Clinical trials are now ongoing to evaluate the effectiveness of tol-DCs to treat rheumatoid arthritis and multiple sclerosis [96]. One of the limitations of inducing tolerance with DCs in autoimmune diseases is selecting the proper immunodominant autoantigen because autoimmune disease antigens are abundant [94]. In the setting of FA, antigens are limited, so targeting DCs for immunotherapy in PA is a very promising alternative.

Oral tolerance to food is a result of complicated interactions among antigens from food intake, the intestinal microbiome,

and specialized DCs and lymphocytes found in the GALT. Failure to develop tolerance or breakdown in tolerance leading to FA can occur at multiple points. Future progress in our understanding of oral tolerance in human subjects should offer crucial insights into the factors that determine the development of natural or therapy-induced long-lasting tolerance.

Key issues

- The prevalence of peanut allergy (PA) has increased worldwide, affecting about 1.1% of children in Western countries.
- PA may induce a broad spectrum of allergic symptoms, from mild to fatal anaphylactic reactions.
- There is no treatment for PA and avoidance of its consumption remains the main therapeutic option.
- Achievement of peanut tolerance is an active process that occurs in the intestinal mucosa, specifically in GALT.
- Intestinal dendritic cells (DCs) have a key function in the immune response to food allergens, directing the response towards tolerance or allergy.
- There are different factors that affect the tolerogenic phenotype of DCs, such as the presence of retinoic acid (RA) and vitamin D (VD), the intestinal environment, the diversity of the gut microbiota, and the pro-inflammatory signals of peanut allergens.
- Due to the pivotal role of DCs in the development of PA, this cell type is interesting as a therapeutic target for the development of new therapeutic strategies.

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Declaration of interest

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Sicherer SH, Sampson HA. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol.* **2014** Feb;133(2):291–307. quiz 308.

2. Koplin JJ, Mills EN, Allen KJ. Epidemiology of food allergy and food-induced anaphylaxis: is there really a Western world epidemic? *Curr Opin Allergy Clin Immunol.* **2015** Oct;15(5):409–416. PubMed PMID: 26258921; eng.
3. Nwaru BI, Hickstein L, Panesar SS, et al. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy.* **2014** Aug;69(8):992–1007. PubMed PMID: 24816523; eng.
4. Sicherer SH, Munoz-Furlong A, Godbold JH, et al. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. *J Allergy Clin Immunol.* **2010** Jun;125(6):1322–1326. PubMed PMID: 20462634; eng.
5. Spergel JM, Beausoleil JL, Pawlowski NA. Resolution of childhood peanut allergy. *Ann Allergy Asthma Immunol.* **2000** Dec;85(6 Pt 1):473–476. PubMed PMID: 11152168; eng.
6. Grundy J, Matthews S, Bateman B, et al. Rising prevalence of allergy to peanut in children: data from 2 sequential cohorts. *J Allergy Clin Immunol.* **2002** Nov;110(5):784–789. PubMed PMID: 12417889; eng.
7. Hoyos-Bachilloglu R, Ivanovic-Zuvic D, Alvarez J, et al. Prevalence of parent-reported immediate hypersensitivity food allergy in Chilean school-aged children. *Allergol Immunopathol (Madr).* **2014** Nov-Dec;42(6):527–532. PubMed PMID: 24388812; eng.
8. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol.* **2001** Jan;107(1):191–193. PubMed PMID: 11150011; eng.
9. Berin MC. Mechanisms of allergic sensitization to foods: bypassing immune tolerance pathways. *Immunol Allergy Clin North Am.* **2012** Feb;32(1):1–10. PubMed PMID: 22244229; eng.
10. Ruiter B, Shreffler WG. The role of dendritic cells in food allergy. *J Allergy Clin Immunol.* **2012** Apr;129(4):921–928. PubMed PMID: 22464669; eng.
11. Lack G, Fox D, Northstone K, et al. Factors associated with the development of peanut allergy in childhood. *N Engl J Med.* **2003** Mar 13;348(11):977–985. PubMed PMID: 12637607; eng.
12. Jin H, Oyoshi MK, Le Y, et al. IL-21R is essential for epicutaneous sensitization and allergic skin inflammation in humans and mice. *J Clin Invest.* **2009**;119:47–60.
13. Du Toit G, Roberts G, Sayre PH, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med.* **2015** Feb 26;372(9):803–813. PubMed PMID: 25705822; PubMed Central PMCID: PMC4416404. eng.
14. Lorenz AR, Scheurer S, Vieths S. Food allergens: molecular and immunological aspects, allergen databases and cross-reactivity. *Chem Immunol Allergy.* **2015**;101:18–29. PubMed PMID: 26022861; eng.
15. Becker WM, Jappe U. Peanut allergens. *Chem Immunol Allergy.* **2014**;100:256–267. PubMed PMID: 24925406; eng.
16. Petersen A, Kull S, Rennert S, et al. Peanut defensins: novel allergens isolated from lipophilic peanut extract. *J Allergy Clin Immunol.* **2015** Nov;136(5):1295–301.e1-5. PubMed PMID: 26037551; eng.
17. Schwager C, Kull S, Behrends J, et al. Peanut oleosins associated with severe peanut allergy-importance of lipophilic allergens for comprehensive allergy diagnostics. *J Allergy Clin Immunol.* **2017** Nov;140(5):1331–1338.e8. PubMed PMID: 28342912; eng.
18. Zhuang Y, Dreskin SC. Redefining the major peanut allergens. *Immunol Res.* **2013** Mar;55(1–3):125–134. PubMed PMID: 22948807; PubMed Central PMCID: PMC4451826. eng.
19. Koppelman SJ, Wensing M, Ertmann M, et al. Relevance of Ara h 1, Ara h 2 and Ara h 3 in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h 2 is the most important peanut allergen. *Clin Exp Allergy.* **2004** Apr;34(4):583–590. PubMed PMID: 15080811; eng.
20. Klemans RJ, Liu X, Knulst AC, et al. IgE binding to peanut components by four different techniques: Ara h 2 is the most relevant in peanut allergic children and adults. *Clin Exp Allergy.* **2013** Aug;43(8):967–974. PubMed PMID: 23889250; eng.
21. Vereda A, van Hage M, Ahlstedt S, et al. Peanut allergy: clinical and immunologic differences among patients from 3 different

- geographic regions. *J Allergy Clin Immunol.* **2011** Mar;127(3):603–607. PubMed PMID: 21093026; eng.
22. Strobel S, Mowat AM. Oral tolerance and allergic responses to food proteins. *Curr Opin Allergy Clin Immunol.* **2006** Jun;6(3):207–213. PubMed PMID: 16670516; eng.
 23. Berin MC, Shreffler WG. Mechanisms underlying induction of tolerance to foods. *Immunol Allergy Clin North Am.* **2016** Feb;36(1):87–102. PubMed PMID: 26617229; eng.
 24. Esterhazy D, Loschko J, London M, et al. Classical dendritic cells are required for dietary antigen-mediated induction of peripheral Treg cells and tolerance. *Nat Immunol.* **2016** May;17(5):545–555. PubMed PMID: 27019226; PubMed Central PMCID: PMC4837106. eng.
 25. Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol.* **2003** Apr;3(4):331–341. PubMed PMID: 12669023; eng.
 26. Chintrajah RS, Hernandez JD, Boyd SD, et al. Molecular and cellular mechanisms of food allergy and food tolerance. *J Allergy Clin Immunol.* **2016** Apr;137(4):984–997. PubMed PMID: 27059726; PubMed Central PMCID: PMC45030841. eng.
 27. Steinman RM. Decisions about dendritic cells: past, present, and future. *Annu Rev Immunol.* **2012**;30:1–22. PubMed PMID: 22136168; eng.
 28. Groux H, Fournier N, Cottrez F. Role of dendritic cells in the generation of regulatory T cells. *Semin Immunol.* **2004** Apr;16(2):99–106. PubMed PMID: 15036233; eng.
 29. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol.* **2007** Jan;7(1):19–30. PubMed PMID: 17170756; eng.
 30. Kushwah R, Hu J. Complexity of dendritic cell subsets and their function in the host immune system. *Immunology.* **2011** Aug;133(4):409–419. PubMed PMID: 21627652; PubMed Central PMCID: PMC3143352. eng.
 31. Varol C, Vallon-Eberhard A, Elinav E, et al. Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity.* **2009** Sep;31(3):502–512. PubMed PMID: 19733097; eng.
 32. Scott CL, Bain CC, Wright PB, et al. CCR2(+)/CD103(-) intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. *Mucosal Immunol.* **2015** Mar;8(2):327–339. PubMed PMID: 25138666; PubMed Central PMCID: PMC4270738. eng.
 33. Becker M, Guttler S, Bachem A, et al. Ontogenic, phenotypic, and functional characterization of XCR1(+) dendritic cells leads to a consistent classification of intestinal dendritic cells based on the expression of XCR1 and SIRPalpha. *Front Immunol.* **2014**;5:326. PubMed PMID: 25120540; PubMed Central PMCID: PMC4112810. eng.
 34. Siddiqui KR, Laffont S, Powrie F. E-cadherin marks a subset of inflammatory dendritic cells that promote T cell-mediated colitis. *Immunity.* **2010** Apr;32(4):557–567. PubMed PMID: 20399121; PubMed Central PMCID: PMC2938478. eng.
 35. Smit JJ, Bol-Schoenmakers M, Hassing I, et al. The role of intestinal dendritic cells subsets in the establishment of food allergy. *Clin Exp Allergy.* **2011** Jun;41(6):890–898. PubMed PMID: 21477183; eng.
 36. Niess JH, Brand S, Gu X, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science.* **2005** Jan 14;307(5707):254–258. PubMed PMID: 15653504; eng.
 37. Hadis U, Wahl B, Schulz O, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity.* **2011** Feb 25;34(2):237–246. PubMed PMID: 21333554; English.
 38. Cassani B, Villablanca EJ, Quintana FJ, et al. Gut-tropic T cells that express integrin alpha4beta7 and CCR9 are required for induction of oral immune tolerance in mice. *Gastroenterology.* **2011** Dec;141(6):2109–2118. PubMed PMID: 21925467; PubMed Central PMCID: PMC3222333. eng.
 39. Akdis CA, Akdis M. Mechanisms of immune tolerance to allergens: role of IL-10 and Tregs. *J Clin Invest.* **2014** Nov;124(11):4678–4680. PubMed PMID: 25365074; PubMed Central PMCID: PMC4347251. eng.
 40. Smaldini PL, Orsini Delgado ML, Fossati CA, et al. Orally-induced intestinal CD4+ CD25+ FoxP3+ Treg controlled undesired responses towards oral antigens and effectively dampened food allergic reactions. *PLoS One.* **2015**;10(10):e0141116. PubMed PMID: 26517875; PubMed Central PMCID: PMC4627767. eng.
 41. Wang M, Yang IV, Davidson EJ, et al. Forkhead box protein 3 demethylation is associated with tolerance induction in peanut-induced intestinal allergy. *J Allergy Clin Immunol.* **2017** May. DOI:10.1016/j.jaci.2017.04.020 PubMed PMID: 28479331; PubMed Central PMCID: PMC5671381. eng.
 42. Cassani B, Villablanca EJ, De Calisto J, et al. Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. *Mol Aspects Med.* **2012** Feb;33(1):63–76. PubMed PMID: 22120429; PubMed Central PMCID: PMC3246074.
 43. Bono MR, Tejon G, Flores-Santibañez F, et al. Retinoic acid as a modulator of T cell immunity. *Nutrients.* **2016** Jun;8(6). PubMed PMID: 27304965; PubMed Central PMCID: PMC4924190. eng. DOI:10.3390/nu8060349
 44. Czarnecki P, Das S, Parigi SM, et al. Retinoic acid and its role in modulating intestinal innate immunity. *Nutrients.* **2017** Jan;9(1). PubMed PMID: 28098786; PubMed Central PMCID: PMC5295112. eng. DOI:10.3390/nu9010068
 45. Bakdash G, Vogelpoel LT, van Capel TM, et al. Retinoic acid primes human dendritic cells to induce gut-homing, IL-10-producing regulatory T cells. *Mucosal Immunol.* **2015** Mar;8(2):265–278. PubMed PMID: 25027601; eng.
 46. Bernardo D, Mann ER, Al-Hassi HO, et al. Lost therapeutic potential of monocyte-derived dendritic cells through lost tissue homing: stable restoration of gut specificity with retinoic acid. *Clin Exp Immunol.* **2013** Oct;174(1):109–119. PubMed PMID: 23607934; PubMed Central PMCID: PMC3784218. eng.
 47. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med.* **2007** Aug 6;204(8):1757–1764. PubMed PMID: 17620361; PubMed Central PMCID: PMC2118683. eng.
 - **This study demonstrates that intestinal CD103+ DCs induce the development of Foxp3+ Tregs in a TGF-β-dependent and a retinoic acid-dependent manner.**
 48. Belkaid Y, Oldenhove G. Tuning microenvironments: induction of regulatory T cells by dendritic cells. *Immunity.* **2008** Sep;29(3):362–371. PubMed PMID: 18799144; PubMed Central PMCID: PMC23415213. eng.
 49. Dawicki W, Li C, Town J, et al. Therapeutic reversal of food allergen sensitivity by mature retinoic acid-differentiated dendritic cell induction of LAG3+CD49b-Foxp3- regulatory T cells. *J Allergy Clin Immunol.* **2017** May;139(5):1608–1620.e3. PubMed PMID: 28277274; eng.
 50. Hall JA, Cannons JL, Grainger JR, et al. Essential role for retinoic acid in the promotion of CD4(+) T cell effector responses via retinoic acid receptor alpha. *Immunity.* **2011** Mar 25;34(3):435–447. PubMed PMID: 21419664; PubMed Central PMCID: PMC3415227. eng.
 51. Christakos S, Dhawan P, Verstuyf A, et al. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev.* **2016** Jan;96(1):365–408. PubMed PMID: 26681795; PubMed Central PMCID: PMC4839493. eng.
 52. Allen KJ, Koplin JJ, Ponsonby AL, et al. Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J Allergy Clin Immunol.* **2013** Apr;131(4):1109–16, 1116.e1–6. PubMed PMID: 23453797; eng.
 53. Mullins RJ, Clark S, Wiley V, et al. Neonatal vitamin D status and childhood peanut allergy: a pilot study. *Ann Allergy Asthma Immunol.* **2012** Nov;109(5):324–328. PubMed PMID: 23062387; eng.
 54. Szeles L, Keresztes G, Torocsik D, et al. 1,25-dihydroxyvitamin D3 is an autonomous regulator of the transcriptional changes leading to a tolerogenic dendritic cell phenotype. *J Immunol.* **2009** Feb 15;182(4):2074–2083. PubMed PMID: 19201860; eng.
 55. Penna G, Adorini L. 1 alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells

- leading to impaired alloreactive T cell activation. *J Immunol.* 2000 Mar 1;164(5):2405–2411. [ji_v164n5p2405 \[pii\]](#). PubMed PMID: 10679076; eng.
- **This study demonstrates that VD inhibits the maturation and differentiation of human DCs in vitro, leading to the induction of T-cell hyporesponsiveness.**
56. Manavalan JS, Rossi PC, Vlad G, et al. High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. *Transpl Immunol.* 2003 Jul-Sep;11(3–4):245–258. PubMed PMID: 12967778; eng.
 57. van der Aar AM, Sibiryak DS, Bakdash G, et al. Vitamin D3 targets epidermal and dermal dendritic cells for induction of distinct regulatory T cells. *J Allergy Clin Immunol.* 2011 Jun;127(6):1532–40 e7. S0091-6749(11)00363-0[pii]. PubMed PMID: 21497886; eng.
 58. Bak NF, Bendix M, Hald S, et al. High-dose vitamin D3 supplementation decreases the number of colonic CD103+ dendritic cells in healthy subjects. *Eur J Nutr.* 2017 Sep. DOI:10.1007/s00394-017-1531-y PubMed PMID: 28913556; eng
 59. Marietta E, Rishi A, Taneja V. Immunogenetic control of the intestinal microbiota. *Immunology.* 2015 Jul;145(3):313–322. PubMed PMID: 25913295; PubMed Central PMCID: PMC4479531. eng.
 60. Rachid R, Chatila TA. The role of the gut microbiota in food allergy. *Curr Opin Pediatr.* 2016 Dec;28(6):748–753. PubMed PMID: 27749359; eng.
 61. Mazmanian SK, Liu CH, Tzianabos AO, et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell.* 2005 Jul;122(1):107–118. PubMed PMID: 16009137; eng.
 62. Kozakova H, Schwarzer M, Tuckova L, et al. Colonization of germ-free mice with a mixture of three lactobacillus strains enhances the integrity of gut mucosa and ameliorates allergic sensitization. *Cell Mol Immunol.* 2016 Mar;13(2):251–262. PubMed PMID: 25942514; PubMed Central PMCID: PMC4786630. eng.
 63. Noval Rivas M, Burton OT, Wise P, et al. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol.* 2013 Jan;131(1):201–212. PubMed PMID: 23201093; PubMed Central PMCID: PMC3860814. eng.
 64. Ling Z, Li Z, Liu X, et al. Altered fecal microbiota composition associated with food allergy in infants. *Appl Environ Microbiol.* 2014 Apr;80(8):2546–2554. PubMed PMID: 24532064; PubMed Central PMCID: PMC3993190. eng.
 65. Azad MB, Konya T, Guttman DS, et al. Infant gut microbiota and food sensitization: associations in the first year of life. *Clin Exp Allergy.* 2015 Mar;45(3):632–643. PubMed PMID: 25599982; eng.
 - **This study provides evidence for early-life dysbiosis and its association with food sensitization.**
 66. Hua X, Goedert JJ, Pu A, et al. Allergy associations with the adult fecal microbiota: analysis of the American Gut Project. *EBioMedicine.* 2016 Jan;3:172–179. PubMed PMID: 26870828; PubMed Central PMCID: PMC4739432. eng.
 67. Stefka AT, Feehley T, Tripathi P, et al. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U S A.* 2014 Sep;111(36):13145–13150. PubMed PMID: 25157157; PubMed Central PMCID: PMC4246970. eng.
 68. Dupont C. Food allergy: recent advances in pathophysiology and diagnosis. *Ann Nutr Metab.* 2011;59(Suppl 1):8–18. PubMed PMID: 22189252; eng.
 69. Dreskin SC. Do HLA genes play a role in the genetics of peanut allergy? *Ann Allergy Asthma Immunol.* 2006;96:766–768. United States.
 70. Chambers SJ, Bertelli E, Winterbone MS, et al. Adoptive transfer of dendritic cells from allergic mice induces specific immunoglobulin E antibody in naive recipients in absence of antigen challenge without altering the T helper 1/T helper 2 balance. *Immunology.* 2004 May;112(1):72–79. PubMed PMID: 15096186; PubMed Central PMCID: PMC1782460. eng.
 - **This study provides evidence of the pivotal role of DCs in the development of food allergy in a murine model of cow's milk allergy.**
 71. Frischmeyer-Guerrero PA, Guerrero AL, Chichester KL, et al. Dendritic cell and T cell responses in children with food allergy. *Clin Exp Allergy.* 2011 Jan;41(1):61–71. PubMed PMID: 20825428; PubMed Central PMCID: PMC3006008. eng.
 - **This study shows that human CD4s of children with cow's milk allergy produce higher levels of Th2 cytokines than CD4s of control subjects.**
 72. Snider DP, Marshall JS, Perdue MH, et al. Production of IgE antibody and allergic sensitization of intestinal and peripheral tissues after oral immunization with protein Ag and cholera toxin. *J Immunol.* 1994 Jul 15;153(2):647–657. PubMed PMID: 8021502; eng.
 73. Gagliardi MC, Sallusto F, Marinaro M, et al. Cholera toxin induces maturation of human dendritic cells and licenses them for Th2 priming. *Eur J Immunol.* 2000 Aug;30(8):2394–2403. PubMed PMID: 10940931; eng.
 74. Blázquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol.* 2008 Apr 1;180: 4441–4450.
 75. Ruter B, Shreffler WG. Innate immunostimulatory properties of allergens and their relevance to food allergy. *Semin Immunopathol.* 2012 Sep;34(5):617–632. PubMed PMID: 22886110; PubMed Central PMCID: PMC3459673. eng.
 76. Shreffler WG, Castro RR, Kucuk ZY, et al. The major glycoprotein allergen from *Arachis hypogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. *J Immunol.* 2006 Sep 15;177(6):3677–3685. PubMed PMID: 16951327; eng.
 77. Maleki SJ, Chung SY, Champagne ET, et al. The effects of roasting on the allergenic properties of peanut proteins. *J Allergy Clin Immunol.* 2000 Oct;106(4):763–768. PubMed PMID: 11031348; eng.
 78. Abramson MJ, Puy RM, Weiner JM. Injection allergen immunotherapy for asthma. *Cochrane Database Syst Rev.* 2010 Aug 4;(8): CD001186. DOI: 10.1002/14651858.CD001186.pub2. PubMed PMID: 20687065; eng.
 79. Calamita Z, Saconato H, Pela AB, et al. Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. *Allergy.* 2006 Oct;61(10):1162–1172. PubMed PMID: 16942563; eng.
 80. Boyle RJ, Elremeli M, Hockenhull J, et al. Venom immunotherapy for preventing allergic reactions to insect stings. *Cochrane Database Syst Rev.* 2012 Oct;17(10):CD008838. PubMed PMID: 23076950; eng.
 81. Meadows A, Kaambwa B, Novielli N, et al. A systematic review and economic evaluation of subcutaneous and sublingual allergen immunotherapy in adults and children with seasonal allergic rhinitis. *Health Technol Assess.* 2013 Jul;17(27):vi, xi–xiv, 1–322. PubMed PMID: 23827204; PubMed Central PMCID: PMC4780904. eng.
 82. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol.* 2014 Mar 1;133(3):621–631. PubMed PMID: 24581429; English.
 83. Wood RA. Food allergen immunotherapy: current status and prospects for the future. *J Allergy Clin Immunol.* 2016 Apr;137(4):973–982. PubMed PMID: 27059725; eng.
 84. Jones SM, Agbotounou WK, Fleischer DM, et al. Safety of epicutaneous immunotherapy for the treatment of peanut allergy: a phase 1 study using the Viaskin patch. *J Allergy Clin Immunol.* 2016 Apr;137(4):1258–1261.e10. PubMed PMID: 26920463; eng.
 85. Gernez Y, Nowak-Węgrzyn A. Immunotherapy for food allergy: are we there yet? *J Allergy Clin Immunol Pract.* 2017 Mar-Apr;5(2):250–272. PubMed PMID: 28283151; eng.
 - **This is an excellent review article on the evidence of the clinical outcomes and the underlying mechanisms of the effect of current immunotherapies tested for FA.**
 86. Weissler KA, Rasooly M, DiMaggio T, et al. Identification and analysis of peanut-specific effector T and regulatory T cells in children allergic and tolerant to peanut. *J Allergy Clin Immunol.* 2018 Feb. DOI:10.1016/j.jaci.2018.01.035 PubMed PMID: 29454004; eng
 87. Frischmeyer-Guerrero PA, Keet CA, Guerrero AL, et al. Modulation of dendritic cell innate and adaptive immune functions by oral and sublingual immunotherapy. *Clin Immunol.* 2014 Nov;155(1):47–59.

- PubMed PMID: 25173802; PubMed Central PMCID: PMC4252363. eng.
88. Syed A, Garcia MA, Lyu SC, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol.* **2014** Feb;133(2):500–510. PubMed PMID: 24636474; PubMed Central PMCID: PMC4121175. eng.
 89. Wisniewski JA, Commins SP, Agrawal R, et al. Analysis of cytokine production by peanut-reactive T cells identifies residual Th2 effectors in highly allergic children who received peanut oral immunotherapy. *Clin Exp Allergy.* **2015** Jul;45(7):1201–1213. PubMed PMID: 25823600; PubMed Central PMCID: PMC4472497. eng.
 90. Tordesillas L, Mondoulet L, Blazquez AB, et al. Epicutaneous immunotherapy induces gastrointestinal LAP(+) regulatory T cells and prevents food-induced anaphylaxis. *J Allergy Clin Immunol.* **2017** Jan;139(1):189–201.e4. PubMed PMID: 27417020; PubMed Central PMCID: PMC45149454. eng.
 91. Sampson HA, Shreffler WG, Yang WH, et al. Effect of varying doses of epicutaneous immunotherapy vs placebo on reaction to peanut protein exposure among patients with peanut sensitivity: a randomized clinical trial. *Jama.* **2017** Nov 14;318(18):1798–1809. PubMed PMID: 29136445; PubMed Central PMCID: PMC5820709. eng.
 92. Versteven M, Van den Bergh MJM, Marcq E, et al. Dendritic cells and programmed death-1 blockade: a joint venture to combat cancer. *Front Immunol.* **2018**;9:394. PubMed PMID: 29599770; PubMed Central PMCID: PMC5863527. eng.
 93. Hackstein H, Thomson AW. Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. *Nat Rev Immunol.* **2004** Jan;4(1):24–34. PubMed PMID: 14704765; eng.
 94. Pozsgay J, Szekanecz Z, Sármay G. Antigen-specific immunotherapies in rheumatic diseases. *Nat Rev Rheumatol.* **2017** Sep;13(9):525–537. PubMed PMID: 28701761; eng.
 95. Popov I, Li M, Zheng X, et al. Preventing autoimmune arthritis using antigen-specific immature dendritic cells: a novel tolerogenic vaccine. *Arthritis Res Ther.* **2006**;8(5):R141. PubMed PMID: 16911769; PubMed Central PMCID: PMC1779442. eng.
 96. Phillips BE, Garciafigueroa Y, Trucco M, et al. Clinical tolerogenic dendritic cells: exploring therapeutic impact on human autoimmune disease. *Front Immunol.* **2017**;8:1279. PubMed PMID: 29075262; PubMed Central PMCID: PMC5643419. eng.
 97. Benham H, Nel HJ, Law SC, et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. *Sci Transl Med.* **2015** Jun 3;7(290):290ra87. PubMed PMID: 26041704; eng.
 98. Borzutzky A, Camargo CA Jr. Role of vitamin D in the pathogenesis and treatment of atopic dermatitis. *Expert Rev Clin Immunol.* **2013** Aug;9(8):751–760. PubMed PMID: 23971753; eng.
 99. Rodriguez B, Prioult G, Bibiloni R, et al. Germ-free status and altered caecal subdominant microbiota are associated with a high susceptibility to cow's milk allergy in mice. *FEMS Microbiol Ecol.* **2011** Apr;76(1):133–144. PubMed PMID: 21223329; eng.
 100. Kamalakannan M, Chang LM, Grishina G, et al. Identification and characterization of DC-SIGN-binding glycoproteins in allergenic foods. *Allergy.* **2016** Aug;71(8):1145–1155. PubMed PMID: 26948687; eng.