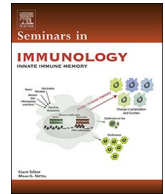




Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Review

Oral tolerance and allergy

Ahmad Hamad^{a,*}, Wesley Burks^b^a University of North Carolina, Chapel Hill, NC Thurston Arthritis Research Center, UNC Chapel Hill 3300 Thurston Building, Chapel Hill, NC 27599, United States^b University of North Carolina, Chapel Hill, NC 4032 Bondurant Hall, Campus Box 7000, Chapel Hill, NC 27599-7000, United States

ARTICLE INFO

Keywords:

Food allergy
Oral tolerance
Oral immunotherapy
Sustained unresponsiveness

ABSTRACT

Food allergy prevalence is increasing in the developed world. It's estimated that each year in the US, anaphylaxis to food results in 30,000 emergency room visits and 150 deaths. Over the past few decades, there has been a tremendous effort to better understand the pathogenesis of food allergy and mechanisms of food tolerance. In this article we review the structural of the gastrointestinal immune system and mechanisms of natural tolerance to food. We then review the factors that may result in the IgE mediated hypersensitivity reaction to food allergens resulting in clinical food allergy. Lastly, we provide a brief review of the efforts to induce immune tolerance to patients with food allergy.

1. Introduction

Food allergy is believed to be rising in developed countries with prevalence estimations more than 1–2% but less than 10% of the general population [1]. Still, most people are tolerant to food antigens which makes it reasonable to think of food allergy as a failure in the inherent mechanisms of oral tolerance. A lot of work has been done to answer the question why certain people are allergic to certain food allergens. Although there is still much to be learned about tolerance and food allergy, there is enough evidence to suggest that food allergy or failure of oral tolerance is a multifactorial issue. Genetics, food allergenicity, food processing, environmental factors, microbiota, and other, yet undiscovered, elements are all possible contributing factors.

Food allergy is defined as an immune mediated reaction to a food allergen that is mediated by IgE antibodies. Food allergens were classically thought of as glycoproteins and reproducibility upon re-exposure was seen as a hallmark for diagnosis but that understanding has been challenged by the recent discovery of mammalian meat allergy. Food intolerance is a term that is usually used for non-allergic (non-immune) adverse reactions to food and symptoms of food intolerance are typically limited to the digestive system. Food tolerance can be defined as a state of immune unresponsiveness to food antigens regardless of continuous exposure that can be mediated by deletion, anergy or induction of regulatory T cells. It's important to notice that we talk of oral tolerance to food not as an opposite of food intolerance but an opposite of food allergy. A person may not be allergic to milk but still intolerant to it e.g. due to lactase insufficiency.

In this review article, will discuss the current understanding of

mechanisms of natural immune tolerance to food. Then we will discuss the factors contributing to oral tolerance failure or developing of allergy. Lastly, we will review the current attempts to induce food tolerance in patients with food allergy.

2. Structure of the gastrointestinal immune system

To understand the interaction between food as foreign antigens and our immune system, we need to understand the features of the immune system in the gastrointestinal tract. The gastrointestinal immune system is the largest and most complex mucosal immune system (other systems are cutaneous, bronchopulmonary and genitourinary).

In the gastrointestinal tract there is a single outer epithelial layer sitting on a basement membrane with underlying connective tissue (lamina propria) that contains blood vessels, lymphatic vessels and lymphoid tissue. Beneath the lamina propria is a sub mucosal layer surrounded by layers of smooth muscles (Fig. 1).

In the epithelial layer there are different types of intestinal epithelial cells: mucus-secreting goblet cells, absorptive epithelial cells, antigen-sampling M cells, and anti-bacterial peptide secreting Paneth cells [2].

In the lamina propria there are different cells of the lymphoid tissue involved in innate and adaptive immunity: macrophages, dendritic cells, neutrophils and innate lymphoid cells are mainly involved in innate immunity and communicate with T cells and B cells that are involved mainly in adaptive immunity. These cells mainly involved in adaptive immunity are organized either as collections of lymphocytes in close association with the epithelial layer (MALT) or in lymphoid nodes

* Corresponding author.

E-mail addresses: Ahmad.hamad@unchealth.unc.edu (A. Hamad), wburks@email.unc.edu (W. Burks).<http://dx.doi.org/10.1016/j.ssmim.2017.07.001>

Received 16 August 2016; Accepted 11 July 2017

1044-5323/ © 2017 Elsevier Ltd. All rights reserved.

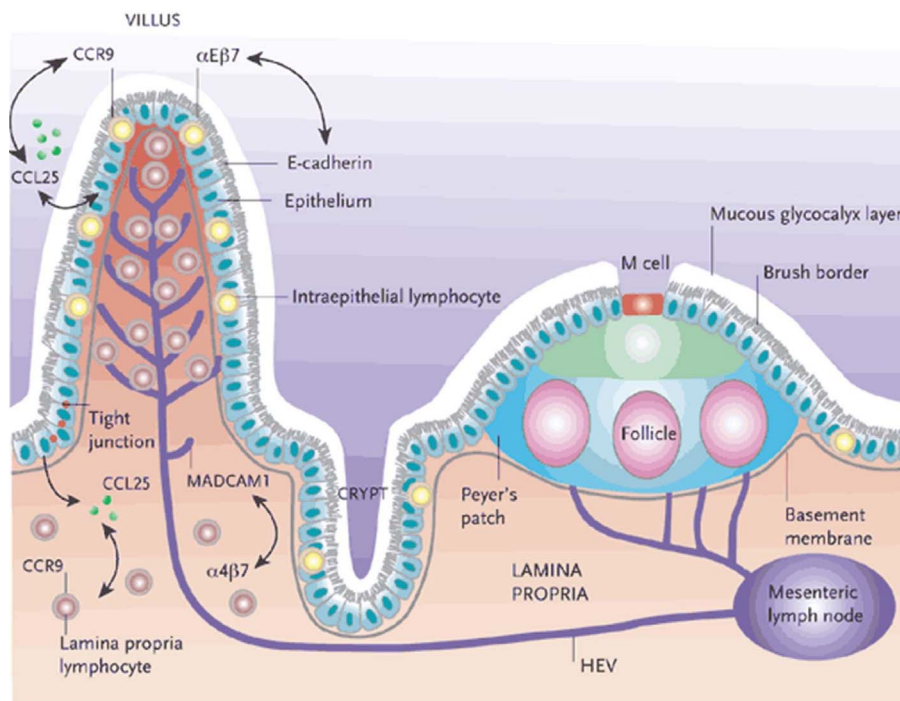


Fig. 1. The gastrointestinal tract is lined by an epithelial layer which contains M cells that sample antigens from the gastrointestinal lumen and deliver them to antigen presenting cells in lamina propria. Some dendritic cells in lamina propria also extended dendrites into the lumen to uptake antigens. Antigen presenting cells present processed antigens to T cells in Peyer's patches and mesenteric lymph nodes. T cells home back to lamina propria as CCR9 which is expressed by almost all lymphocytes of the small intestine, is a receptor for the chemokine CCL25 which is produced by epithelial cells of the small intestine. iTreg migrate to CXCL13-expressing B cell follicles because of up regulation of their CXCR5 receptors. Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Immunology] (Acquired and natural memory T cells join forces at the mucosal front line), copyright (2004).

that get lymphatic vessels from lamina propria (e.g., 100–150 mesenteric lymph nodes). The most prominent MALT anatomic structure is Peyer's patches in the distal ileum beside small aggregates of lymphoid follicles in the appendix and colon. Lingual and palatine tonsils are another organized part of MALT and they get antigens delivered from the oral cavity. They are distinct in that they are separated from the oral cavity by layers of squamous epithelial cells rather than one epithelial cell layer like in Peyer's patches. In our review we will focus on the intestinal lymphoid tissue. These lymphoid follicles in MALT have germinal centers containing B lymphocytes, follicular helper T cells, follicular dendritic cells and macrophages. The germinal centers are surrounded by naïve follicular B cells. Because Peyer's patches are unencapsulated they don't need lymphatic vessels to deliver antigens, unlike germinal centers in the lymph nodes.

The major pathway of antigen delivery is through M cells in epithelium. M cells have large fenestrations in their membrane to enhance uptake of antigens from the gut lumen. They move the antigens through endocytotic vesicles across the cytosol and deliver them by exocytosis at the basement membrane to dendritic cells close to Peyer's patches and lamina propria lymphoid follicles. Also other dendritic cells in lamina propria have extended cytoplasmic processes between the intestinal epithelial cells that sample allergens from the lumen. Then antigen presenting cells present antigens to T cells in MALT or mesenteric lymph nodes. Those naïve T cells develop into effector or regulatory T cells and home back to lamina propria through selective integrins and chemokine receptors. That initial interaction between antigen presenting cells and naïve T cells will induce a certain type of adaptive immune response which will for the most part determine if the person will become tolerant or allergic to that antigen. If naïve T cells develop into Th2 type, they induce B cells to undergo class switching and produce IgE antibodies against those food antigens possibly resulting in clinical food allergy. On that other hand, it's believed that inducing a predominant regulatory T cells response will result in immune tolerance. We assume that tolerance is the rule as it happens in the majority of humans for the majority of food antigens while food allergy is a failure of mechanisms involved in tolerance.

3. Mechanisms of oral tolerance

3.1. Role of dendritic cells

Dendritic cells play a crucial role in immune tolerance as they are needed to present food antigens to naïve T cells. Dendritic cells are the major antigen presenting cells in the lamina propria of gastrointestinal tract. Two types of distinct dendritic cells that express CD11c have been identified in the intestinal mucosa. The first type is CX3CR1(+) and CD103(–) dendritic cells: these cells express the chemokine receptor CX3CR1 which is necessary for extending their dendrites into the lumen of the intestine between the epithelial cells [3,4]. These cells are derived from monocytes under the effect of macrophage colony stimulating factor receptor and Fms-like tyrosine kinase 3 (Flt3) ligands [5]. These cells do not express CCR7 and therefore do not migrate [6]. The other type is CX3CR1(–) and CD103(+) dendritic cells: these cells express CCR7 and thus can migrate in lymph to mesenteric lymph nodes where CCR7 ligands are expressed. Oral tolerance could not be induced in mice deficient in CCR7 ligands which shows the important role of CD103(+) dendritic cells migration in immune tolerance [7].

CX3CR1(+) dendritic cells sample antigens in the intestinal lumen through their dendrites while CD103(+) cells sample antigens by help of M cells and goblet cells as antigens can pass through them from lumen [8] or by help from CX3CR1(+) dendritic cells (and macrophages) via gap junctions [9] Fig. 2.

Viney JL et al. showed that expanding dendritic cells in the intestine by treating mice with growth factor Flt3 ligand (Flt3L) induced systemic tolerance after mice were fed soluble antigen even with very low doses that were ineffective in control animals [10]. Also transferring of dendritic cells from lamina propria in tolerant mice to naïve mice showed to induce a state of hypo-responsiveness after a challenge to ova albumin [11].

3.2. Role of T cells

The mechanisms that affect tolerance to food antigens after that interaction between dendritic cells and naïve T cells are likely similar to the mechanisms involved in peripheral tolerance to other foreign antigens [12]:

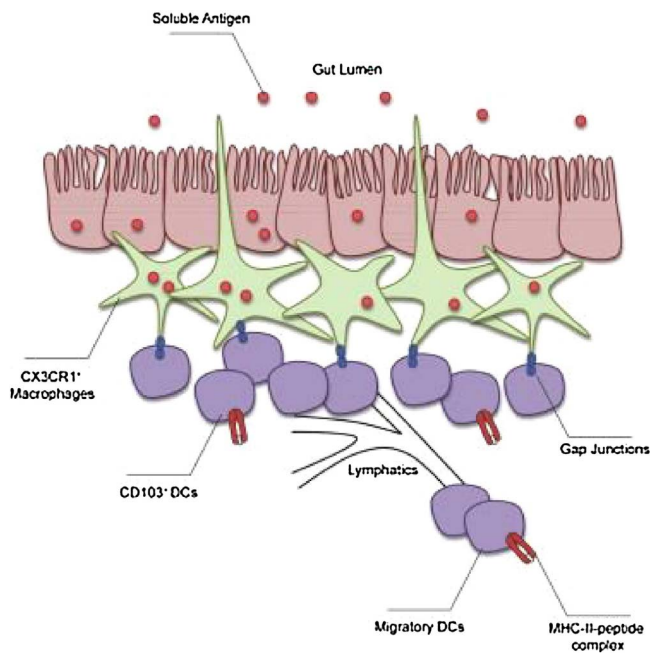


Fig. 2. Sampling of antigens in lumen can occur through dendrites of CX3CR1(+) cells. Goblet cells may uptake small antigens and deliver them to CD103(+) cells in lamina propria (not shown).

Reprinted from, *Immunity*, volume No. 40, Elisa Mazzini, Lucia Massimiliano, Giuseppe Penna, Maria Rescigno, Oral Tolerance Can Be Established via Gap Junction Transfer of Fed Antigens from CX3CR1+ Macrophages to CD103+ Dendritic Cells, Copyright (2014), with permission from Elsevier.

- Deletion (dying by apoptosis): tolerance was shown to be mediated by deletion of antigen specific T cells in mice [13].
- Anergy (incapability of activation by that antigen upon re-exposure): exposure of naive T cells to antigens presented in the absence of co-stimulatory signals is believed to result in inactivation or anergy of the responder cell. Mice with defects in genes involved in anergy of T lymphocytes were not able to achieve oral tolerance [14].
- Suppression by regulatory T cells: It's believed that active suppression or regulatory effect plays a major role in oral tolerance to food antigens as transfer of regulatory CD4+ cells (Treg) or CD8+ cells induced tolerance in naive mice [15]. Several studies showed that Treg transfer may induce oral tolerance in naive mice [16], although there is conflicting data on role of different types of regulatory T cells [17] as different subtypes of T cells were found to have a regulatory function in the gut.

iTreg cells were the first type described of peripheral regulatory T cells. After migration of dendritic cells CD103 (+) to mesenteric lymph nodes they may induce a generation of antigen specific gut-homing CD4+ FoxP3+ regulatory T cells from naive T cells [18]. These are called induced Tregs (iTregs) and express Foxp3. Foxp3 is an important regulatory protein that is necessary to develop induced regulatory T cells. It's important to notice that there are two distinct forms of regulatory CD4T cells: both express Foxp3: those that are induced in thymus are called natural regulatory T cells (nTreg) and those induced in the periphery are called induced regulatory T cells (iTreg cells), so Foxp3 defects should affect both sets of regulatory T cells. Low Foxp3 protein expression was found to develop severe allergic phenotype associated with hyper IgE, eosinophilia, eczema and severe food allergy [19]. Food allergy mediated by Th2 response is believed to be related to defects in the peripheral phenotype. Actually, Josefowicz et al. did show that mice deficient in iTreg cells but with normal numbers of nTreg developed significant Th2 type pathologies in the gastrointestinal and respiratory tracts mucosal surfaces with an increase in IgE levels [20].

Another distinct type of peripheral regulatory CD4+ T cells are called Th3 cells. They were found to play an important role in oral tolerance as well. These cells are characterized by their expression of latency associated peptide (LAP) that binds with TGF- β that could be released from these cells. These cells are induced by oral antigen feeding like iTreg but unlike iTreg they do not express Foxp3 and their mechanism of tolerance is TGF- β dependent. Their secretion of TGF- β seems to induce development of Foxp3+ iTreg [21] so its mechanism of inducing tolerance may not be independent of iTreg after all. This also suggest a role of TGF- β in inducing oral tolerance as was shown in other studies [22].

Gut homing iTreg cells need to undergo proliferation in the lamina propria after they migrate to the gut. This proliferation is induced by CX3CR1(+) macrophages and dendritic cells [23]. CX3CR1(+) macrophages express IL-10 in response to intestinal microbiota which signifies a role of intestinal microbiota and IL-10 in oral tolerance too. Although these iTreg migrate to both mesenteric lymph nodes and MALT (e.g., Peyer's patches [24]) through their expression of CXCR5, mesenteric lymph nodes likely play a more important role in oral tolerance than Peyer's patches. That was suggested when surgical removal of mesenteric lymph nodes showed a failure to develop oral tolerance while removal of Peyer's patches did not [25,26].

Studies have been done to identify the difference in other T cell types in healthy controls and people with food allergy. Th1 phenotype is believed to exist in both groups as peanut specific T cells primarily of Th1 phenotype were detected in groups with or without peanut allergy but only peanut allergic patients had a prominent Th2 profile [27,28]. It's likely that a predominant Th2 response would result in food allergy, while a predominant Treg response would result in oral tolerance. Several factors are important in determining which T cells response will predominate and thereby whether there will be oral tolerance. These will be discussed briefly below and include the role of food characteristics, initial antigen presentation site, the role of adjuvants, the role of microbiota and the role of diet.

4. Factors that affect oral immune tolerance

4.1. Role of food characteristics

Food allergens are not equal in their allergenicity and this may play a key role in inducing immune tolerance or allergy to certain food. Food allergens are known to be derived from either plants or animals. Most of the food allergens were found to be derived from a small number of protein families [29]. Even these food allergens may alter their allergenicity in response to different processing methods of food. For example, roasting peanut has shown to make peanuts more allergenic [30] while heating milk and egg has shown to make them less allergenic [31]. It's hypothesized that processing can change the structure of food antigens to an extent that they can be recognized by antigen presenting cells differently. We also used to believe that food allergens are proteins but this generalization has been changed by the discovery of alpha gal food allergy where patients react to a carbohydrate molecule, galactose-alpha 1,3 galactoses in mammalian meat [32].

4.2. Role of an initial food antigens presentation site

Initial exposure to food antigens through different sites in the body may influence different immune responses. Common sites of allergen exposure include the gastrointestinal tract, skin, and respiratory tract. Antigen presentation in the gastrointestinal tract usually results in oral tolerance. This clinical oral tolerance seems to be mediated mostly by Tregs as noted earlier. However, the gastrointestinal tract in addition to its mucosal immune system that promote tolerance, has many digestive enzymes that may change food antigens prior to their uptake by antigen presenting cells. This may specifically affect conformational epitopes in food making the food more likely to be tolerated. For example, in a

study where mice were fed bovine serum albumin orally they became tolerant but when it was injected in the ileum where it was not degraded by digestive enzymes they were less tolerant to that antigen [33].

Epidemiological studies found that many people with food allergy experienced their first allergic reaction to peanut on their first known ingestion of peanut [34,35] suggesting an initial sensitization through non oral route. That brought up an important question whether initial non-oral site of antigen exposure may play a role in failure of tolerance and development of food allergy. Two routes were suggested: either the sensitization occurred in utero or through skin.

Sensitization through the skin was suggested in a few studies assessing peanut allergy risk and household exposure or using of peanut oil creams [36]. Studies in mice showed sensitization is possible through skin independent of using adjuvants (which is typically used to induce oral sensitization) suggesting that the skin inherently predisposes to sensitization comparing to gastrointestinal mucosa [37–39]. However, other studies showed that mechanical injury is what probably caused dendritic cells in the skin to elicit a Th2 response in absence of an adjuvant [40] and that epicutaneous administration of allergens actually have the capacity to induce desensitization [41] which was confirmed by Mondoulet et al. as they showed that epicutaneous immunotherapy elected reduced or reinforced Th2 systemic response depending on whether skin barrier was intact or disrupted respectively [42]. Therefore, there is a strong argument that sensitization to food through disrupted skin barrier (e.g. eczema) predispose to Th2 response and food allergy [43].

In utero sensitization to allergens occurs by transferring allergens through placenta. There is conflicting data on whether there is an association between maternal ingestion of peanut during pregnancy, which may result in-utero sensitization, and peanut sensitization in children [44,45].

Initial exposure to peanut through breast milk is not an in utero sensitization and is just a form of oral exposure. There is controversy whether food allergens appear in breast milk or induce sensitization in infants [46]. A study on mice showed a possible protective role of food allergen transfer via breast milk [47].

In conclusion, whether that non-oral initial sensitization to food via skin or in-utero truly predisposes to food allergy is still a debatable question and needs further investigation.

4.3. Role of adjuvants

Like in humans, it's believed that the natural response to food antigens in animals is a normal immune response resulting in oral tolerance. In laboratory experiments, one method to break oral tolerance and to induce food sensitivity involves the use of adjuvants. Cholera toxin given orally is a common adjuvant used in mice to induce sensitization when given with food antigens [48,49]. Cholera toxin was found to induce dendritic cells in the lamina propria to mature and migrate to mesenteric lymph nodes and Peyer's patches, prime naive T cells to become Th2 cells [50–52], and induced changes in dendritic cell subsets in the intestinal mucosa [53]. Blazuev and Berin showed that CD 103+ dendritic cells in mesenteric lymph nodes up regulate jagged-2 and OX40L in response to cholera toxin and using anti-OX40L antibodies to neutralize the latter eliminate the cholera toxin induced Th2 cytokine response suggesting that Th2 skewing was mediated by OX40L [54]. Others use superantigen staphylococcus enterotoxin as an adjuvant [55]. Ganeshan et al. showed that oral administration of staphylococcus enterotoxin B caused a decrease in TGF- β and Foxp3 expression in splenocytes [56]. While cholera toxin is unlikely to play a role in food allergy in humans, staphylococcal enterotoxin B produced by some strains of staphylococcus aureus may play a role in food sensitization in humans due to contaminating food or colonizing the skin [57]. Whether food allergy is mediated by undiscovered adjuvants in food or as part of the microbiota is an interesting question that needs further investigation.

4.4. Role of microbiota

Particular bacteria in the gastrointestinal tract like *B. fragilis* and *Clostridium* species were found to enhance regulatory T cells [58,59] and transferring healthy infant microbiota (mainly composed of *Bifidobacterium* and *Bacteroides*) had a protective role against milk sensitization in mice [60]. Rodriguez et al. showed that germ free mice (no microbiome) are more likely to develop sensitization after oral antigen exposure compared to conventional mice [61]. Noval Rivas et al. demonstrated that mice that experienced allergic reactions after oral challenge with food allergen, had a distinct microbiota that differed from allergen-sensitized but non-reactive mice [62]. Transfer of this microbiota to germ-free mice promoted allergen-specific IgE sensitization and allergic reactions after oral challenge which strongly suggest an important role of the gut microbiota in food sensitization and food allergy. One study compared the microbiota of a group of 20 infants with atopic eczema (many with IgE sensitization to foods) to a control group without atopy reported reduced diversity in the microbiome of infants with atopic eczema [63]. These studies suggest a mixed role for microbiota to either enhance sensitization or in other cases to induce tolerance and incited a big interest in its effect on food allergy. A recent study showed that alteration of microbiota in humans (dysbiosis) through antibiotics use, gastric acidity inhibitors, cesarean delivery or fiber diet have been associated with food sensitization and food allergy [64]. Investigators are increasingly interested in the role of probiotics in preventing food allergy or induction of oral tolerance [65] by altering the gut microbiome. One randomized control trial showed that administration of a probiotic and peanut oral immunotherapy was effective in inducing a state of no reactivity after allergen exposure [66].

4.5. Role of diet

4.5.1. Role of vitamin A

CD103+ dendritic cells express high levels of the enzyme RALDH2 (retinal dehydrogenase 2) which converts retinal to retinoic acid (both are compounds of vitamin A). Since CD103+ dendritic cells induce CD4+ Foxp3+ regulatory T cells, this suggest a role for vitamin A in oral tolerance. Also stromal cells of the mesenteric lymph nodes themselves express high levels of retinoic acid and thus are thought to be important for generating gut homing regulator T cells [67]. Vitamin A depleted mice lacked T cells and IgA secreting B cells in the intestinal mucosa but not elsewhere in the body [68]. This suggest a role for vitamin A in inducing effector T cells and B cells too. However, this effect on immunologic mechanism may not reflect a real clinical significance [69].

4.5.2. Role of vitamin D

Like retinoic acid, vitamin D has been suggested to have a role in oral tolerance as it can influence T cells and B cells migration, and has been shown to suppress the development of Th17 cells [70]. Vitamin D is also important for the development of subset of intraepithelial CD8 α -expressing T lymphocytes from the intestinal mucosa [71,72] (CD8 α cells are believed to have a regulatory function). However, the clinical effect of vitamin D on the immune system remains unclear. While a few epidemiological studies suggested a protective role (incidence of food allergy varies with latitude in Australia [73]), other studies linked low vitamin D with elevated sensitization to peanut and shrimp, but not milk or egg [74] giving a mixed picture. One clinical trial actually showed a possible increase risk of food allergy after vitamin D supplementation [75]. In the absence of clinical trials in humans on vitamin D effect in the mechanism or development of oral tolerance, it's hard to make any clinical correlation [76,77].

5. Resolution of food allergy: natural tolerance

Natural history of food allergy may differ between different

Table 1
Summary of oral and sublingual immunotherapy trials.

Study	Type of study	Duration of study	Percent achieved desensitization or sustained unresponsiveness (SU)
	No. of participants	Daily maintenance dose	
	Age of participants		
Peanut OIT			
Blumchen et.al (2010) [100]	Open label N:23 children Age: 3–14 years.	Build up followed by 8 weeks of daily maintenance dose of 500 mg/day followed by 2 weeks of avoidance.	14/22 participants were desensitized to 1 gm peanut (vs.0.19 gm prior to treatment)
Jones et al. [89]	Open label N: 39	Maintenance dose 300 mg peanut protein followed by OFC in 12 months. Then a daily OIT dose of 1800 mg peanut protein. Total 36 months.	Overall 93% desensitized to 3900 mg peanut protein.
Varshney et al. (2011) [101]	RCT N: 28 Age: 1–16 years.	Build up (44 weeks) followed by daily maintenance dose of 4000 mg for one month. Challenge at 48 weeks.	16/19 passed 5000 mg challenge (desensitized) (vs. 280 mg in placebo group)
Anagnostnou et al. (2014)[102]	RCT N:99 Age: 7–16 years.	Treatment group in first phase reached daily dose at 800 mg/day for total of 26 weeks Treatment group in second phase were the control group in the first phase.	62% desensitized to 1400 mg protein in first phase (vs. 0% in placebo) 54% desensitized to 1400 mg protein in second phase treatment group.
Vickery et al. (2014) [103]	Open label N: 39 (24 completed protocol) Age: 1–16 years.	Maintenance dose of 4000 mg peanut protein/day Duration: up to 5 years.	50% passed challenge for 5000 mg peanut protein at 1 month after stopping OIT (SU)
Egg OIT			
Buchanan et al. (2007) [104]	open label N: 7	Duration: 24 months. Maintenance daily dose: 300 mg powdered egg.	57% desensitized to 8 gm of egg protein. 28% achieved SU when challenged in 3–4 months after OIT.
Burks et al. [88]	RCT N: 55 Age: 5–11 years.	Duration: 22 months Maintenance dose: up to 2 g of egg white powder/day.	75% desensitized to 10 g of egg white powder and 28% achieved SU after (4–6 weeks) (Placebo 0% desensitized)
Caminiti et al. (2015) [105]	RCT N: 31	Duration: 4 months followed by OFC. Those desensitized continued another 6 months OIT followed by avoidance for 3 months.	16/17 in treatment arm achieved desensitization and 31% achieved SU at 3 months (vs. 1/14 in placebo group)
Milk OIT			
Longo et al. (2008) [106]	RCT N: 97 age: ≥ 5 years old	Duration: 1 year	36% desensitized became completely tolerant. 54% partly desensitized (5–150 ml) (vs. 0% in placebo).
Skipak et al. (2008) [107]	RCT N: 20 (19 completed) Age: 6–17 years	Maintenance daily dose of 500 mg. Duration: 3–4 months on maintenance.	Median cumulative dose (5140 mg) in treatment group vs. (40 mg) in placebo group.
OIT vs. SLIT (Peanut)			
Narisety et. al (2015) [108]	RCT N: 21 (16 continued) Age: 7–13 years.	Maintenance dose: 3.7 mg/d (SLIT) or 2000 mg/d (OIT) Duration: 6–12 months of maintenance	Threshold of tolerance increase 141 fold in OIT vs. 22 fold in SLIT SU checked after 4 weeks of avoidance. (achieved by 3 participants in OIT vs. 1 participant in SLIT)
OIT vs. SLIT (milk)			
Keet et al. (2012)[109]	RCT (SLIT alone vs. SLIT followed by OIT) N: 30 Age: 6–17 years.	Maintenance dose: 7 mg/d (SLIT alone), 2000 mg/d (OIT alone), 1000 mg/d (SLIT then OIT) Challenge at: 12 then 60 wks.	Participants who passed 8 mg challenge (desensitized): 1/10 in SLIT alone group. 6/10 in SLIT/OIT group. 8/10 in OIT alone group. SU checked at 1 and 6 weeks of avoidance: 3/6 in SLIT/OIT group. 3/8 of OIT alone group
Peanut SLIT			
Fleischer et al. (2013) [110]	RCT N: 40 Age: 12–37 years.	Duration: 44 weeks	70% of SLIT group were desensitized (5 gm challenge) (vs. 15% of placebo)
Burks et al. (2015) [111]	N:40 Age: 12–40 years	Duration: 3 years Minimum daily dose of 165 µg and max of 1386 µg protein daily.	Only 10.8% desensitization to 5 gm peanut protein and they all achieved SU after 8 weeks of avoidance.

OIT: oral immunotherapy, SLIT: sublingual immunotherapy, RCT: randomized control trial, Number, OFC: open food challenge.

allergens. However, we know that a significant percentage of children with milk [78], soy or egg [79] allergy outgrow their allergy [80]. A smaller percentage, about 9%, of patients with tree nut allergy [81] and

21–22% of patients with peanut allergy [82,83] outgrow their allergy as well. Therefore, clinical tolerance can develop spontaneously in many allergic patients.

Most patients who have food allergy and outgrow their allergies experience a rise in IgG4 levels, a decrease in their IgE levels and increase in IL-10 levels [84,85] with no specific trend in serum IgA levels [86]. The mechanisms that underlie this phenomenon is not entirely understood. It's debatable whether it's an active process involving regulatory T cells or a passive process involving loss of Th2 response. But there is evidence that it's more likely than not an active process mediated by Tregs. Several studies showed a higher percentage of allergen specific Tregs cells from patients who outgrew food allergy comparing to those who are still allergic or control patients [31,87]. This strongly suggested that an expansion of allergen-specific Treg cells is associated with the process of outgrowing food allergy.

What is driving that change in Treg proliferation, why it happens in certain people or more likely to happen for certain allergens still needs to be answered. Another important question is: can we intervene and induce oral tolerance in people with food allergy? We will review some of these efforts next.

6. Inducing oral tolerance (immunotherapy)

Immunotherapy is the administration of an allergen in increasing amounts until a maintenance dose is reached then given periodically with goal to achieve a state of unresponsiveness to that allergen. If the allergen is given orally its called oral immunotherapy.

To achieve unresponsiveness temporarily while a patient is receiving immunotherapy is called desensitization. Oral tolerance can be defined clinically as a state of clinical unresponsiveness that persists regardless of allergen exposure. How long should the patient be off immunotherapy before he is declared tolerant or what amount of allergens he should tolerate is undefined yet. Therefore, some experts in the field choose to use "sustained unresponsiveness" instead of tolerance [88] to define a patient who does not react to a certain amount of allergen exposure after a certain duration of avoidance following therapy.

Since immunotherapy was effective clinically in inducing desensitization with or without sustained unresponsiveness, studies were also done to identify immunologic changes induced by different methods of immunotherapy. Because of Tregs role in natural tolerance, there was an interest in detecting immunotherapy effect on Tregs. Studies in animals showed that antigen specific iTreg cells increased while on immunotherapy [89], and were enhanced in mesenteric lymph nodes [90] and others detected changes in FOXP3 methylation [91]. Other immunological biomarkers like levels of food specific IgE and IgG4 were checked as well. Studies almost consistently showed a significant decrease in specific IgE levels with increase IgG4 (similar to trend noticed in natural tolerance) although serum IgE levels tend to become higher initially [92]. It was hypothesized that IgG4 and IgA function as blocking antibodies preventing allergic reaction in sensitized patients [3]. There is a belief that those immunologic effects induced by immunotherapy leading clinically to desensitization or sustained unresponsiveness are temporary while patients receiving therapy [93].

Several factors seem to induce success of immunotherapy in inducing clinical desensitization and sustained unresponsiveness including route of therapy, dose of therapy, duration and frequency of therapy, age of intervention among others [94]. Attempts to induce sustained unresponsiveness have been done both orally and via non-oral route. Non oral routes included: epicutaneous and subcutaneous routes in humans. Trials trying subcutaneous route in the past were aborted due to high rate of systemic effects [95]. Epicutaneous immunotherapy studies are undergoing as well [96]. All routes of immunotherapy seem to induce similar immunological changes although a recent study comparing immune profile in response to oral immunotherapy, sublingual immunotherapy and epicutaneous immunotherapy showed that all immunotherapy routes induce Foxp3+ iTregs but different subsets were induced in each one (OIT and EPIT increased number of LAP+ Tregs) [97]. Oral immunotherapy also seems to be more effective than

epicutaneous immunotherapy or sublingual immunotherapy [98] although side effects and compliance is something to be considered too.

Most studies of food immunotherapy in humans showed that most patients achieve desensitization while on immunotherapy but only a small percentage achieve sustained unresponsiveness. Table 1 below is an attempt to summarize results of several trials done to achieve desensitization or sustained unresponsiveness using different modes of immunotherapy [99]:

7. Conclusion

Thinking of food allergy as a purely Th2 response to food antigens and food tolerance as an active immune response mediated by iTreg is likely an oversimplification of complicated immune mechanisms in the skin or mucosal membranes that have the initial exposure to food allergens. Nevertheless, clinical trials in food allergy in the past decade proved that our understanding of mechanisms of oral tolerance to food may help in preventing food allergy or inducing tolerance after its diagnosis. Current work done to investigate several types of immunotherapy (OIT, SLIT, EPIT, SCIT) shows their potential to induce oral tolerance for variable durations and to variable amounts of food allergens in many patients. Clinically such induced tolerance may reduce their risk of anaphylaxis and mortality, and improve their quality of life. Whether these therapies are ready to be used by clinicians in their daily practice is still under investigation.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgment

Edwin Kim M.D. M.S. for critical reading of the article.

References

- [1] J. Savage, C.B. Johns, Food allergy: epidemiology and natural history, *Immunol. Allergy Clin. North Am.* 35 (1) (2015) 45–59 http://www.ncbi.nlm.nih.gov/pubmed/?term=Burks%20AW%5BAuthor%5D&cauthor=true&cauthor_uid=23534975.
- [2] Abul K. Abbas, Andrew H. Lichtman, *Basic Immunology: Functions and Disorders of the Immune System*, Saunders, Philadelphia, Pa, 2011.
- [3] J.H. Niess, S. Brand, X. Gu, L. Landsman, S. Jung, B.A. McCormick, et al., CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance, *Science* 307 (5707) (2005) 254–258 http://www.ncbi.nlm.nih.gov/pubmed/?term=Burks%20AW%5BAuthor%5D&cauthor=true&cauthor_uid=23534975.
- [4] M. Rescigno, M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, et al., Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria, *Nat. Immunol.* 2 (4) (2001) 361–367 http://www.ncbi.nlm.nih.gov/pubmed/?term=Burks%20AW%5BAuthor%5D&cauthor=true&cauthor_uid=23534975.
- [5] M. Bogunovic, F. Ginhoux, J. Helft, L. Shang, D. Hashimoto, M. Greter, et al., Origin of the lamina propria dendritic cell network, *Immunity* 31 (3) (2009) 513–525.
- [6] O. Schulz, E. Jaensson, E.K. Persson, X. Liu, T. Worbs, W.W. Agace, et al., Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions, *J. Exp. Med.* 206 (13) (2009) 3101–3114.
- [7] T. Worbs, U. Bode, S. Yan, M.W. Hoffmann, G. Hintzen, G. Bernhardt, et al., Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells, *J. Exp. Med.* 203 (3) (2006) 519–527.
- [8] J.R. McDole, L.W. Wheeler, K.G. McDonald, B. Wang, V. Konjufca, K.A. Knoop, et al., Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine, *Nature* 483 (7389) (2012) 345–349.
- [9] E. Mazzini, L. Massimiliano, G. Penna, M. Rescigno, Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1+ macrophages to CD103+ dendritic cells, *Immunity* 40 (2) (2014) 248–261.
- [10] J.L. Viney, A.M. Mowat, J.M. O'Malley, E. Williamson, N.A. Fanger, Expanding dendritic cells in vivo enhances the induction of oral tolerance, *J. Immunol.* 160 (12) (1998) 5815–5825.
- [11] F.G. Chirido, O.R. Millington, H. Beacock-Sharp, A.M. Mowat, Immunomodulatory dendritic cells in intestinal lamina propria, *Eur. J. Immunol.* 35 (6) (2005) 1831–1840.

- [12] M.C. Berin, Can we produce true tolerance in patients with food allergy? *J. Allergy Clin. Immunol.* 131 (1) (2013) 14–22.
- [13] Y. Chen, J. Inobe, R. Marks, P. Gonnella, V.K. Kuchroo, H.L. Weiner, Peripheral deletion of antigen-reactive T cells in oral tolerance, *Nature* 376 (6536) (1995) 177–180.
- [14] M.A. Kriegl, C. Rathinam, R.A. Flavell, E3 ubiquitin ligase GRAIL controls primary T cell activation and oral tolerance, *Proc. Natl. Acad. Sci.* 106 (39) (2009) 16770–16775.
- [15] Y. Chen, J. Inobe, H.L. Weiner, Induction of oral tolerance to myelin basic protein in CD8-depleted mice: both CD4+ and CD8+ cells mediate active suppression, *J. Immunol.* 155 (2) (1995) 910–916.
- [16] H. Yamashita, K. Takahashi, H. Tanaka, H. Nagai, N. Inagaki, Overcoming food allergy through acquired tolerance conferred by transfer of Tregs in a murine model, *Allergy* 67 (2) (2012) 201–209.
- [17] M.M. Rezende, I. Hassing, M. Bol-Schoenmakers, R. Bleumink, L. Boon, J. van Bilsen, et al., CD4+ CD25+ T regulatory cells do not transfer oral tolerance to peanut allergens in a mouse model of peanut allergy, *Clin. Exp. Allergy* 41 (9) (2011) 1324–1333.
- [18] V. Cerovic, S. Houston, C. Scott, A. Aumeunier, U. Yrlid, A. Mowat, et al., Intestinal CD103+ dendritic cells migrate in lymph and prime effector T cells, *Mucosal Immunol.* 6 (1) (2013) 104–113.
- [19] T.R. Torgerson, A. Linane, N. Moes, S. Anover, V. Mateo, F. Rieux-Laucat, et al., Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene, *Gastroenterology* 132 (5) (2007) 1705–1717.
- [20] S.Z. Josefowicz, R.E. Niec, H.Y. Kim, P. Treuting, T. Chinen, Y. Zheng, et al., Extrathymically generated regulatory T cells control mucosal TH2 inflammation, *Nature* 482 (7385) (2012) 395–399.
- [21] Y. Carrier, J. Yuan, V.K. Kuchroo, H.L. Weiner, Th3 cells in peripheral tolerance. I. Induction of Foxp3-positive regulatory T cells by Th3 cells derived from TGF- β T cell-transgenic mice, *J. Immunol.* 178 (1) (2007) 179–185.
- [22] A. Miller, O. Lider, A.B. Roberts, M.B. Sporn, H.L. Weiner, Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of transforming growth factor beta after antigen-specific triggering, *Proc. Natl. Acad. Sci.* 89 (1) (1992) 421–425.
- [23] U. Hadis, B. Wahl, O. Schulz, M. Hardtke-Wolenski, A. Schippers, N. Wagner, et al., Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria, *Immunity* 34 (2) (2011) 237–246.
- [24] J.A. Mattingly, B.H. Waksman, Immunologic suppression after oral administration of antigen I. Specific suppressor cells formed in rat Peyer's patches after oral administration of sheep erythrocytes and their systemic migration, *J. Immunol.* 121 (5) (1978) 1878–1883.
- [25] T.W. Spahn, A. Fontana, A. Faria, A.J. Slavin, H. Eugster, X. Zhang, et al., Induction of oral tolerance to cellular immune responses in the absence of Peyer's patches, *Eur. J. Immunol.* 31 (4) (2001) 1278–1287.
- [26] T.W. Spahn, H.L. Weiner, P.D. Rennett, N. Lügering, A. Fontana, W. Domschke, et al., Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches, *Eur. J. Immunol.* 32 (4) (2002) 1109–1113.
- [27] V. Turcanu, S.J. Maleki, G. Lack, Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts, *J. Clin. Invest.* 111 (7) (2003) 1065–1072.
- [28] A. Flinterman, S. Pasmans, C. den Hartog Jager, M. Hoekstra, C. Bruijnzeel-Koomen, E. Knol, et al., T cell responses to major peanut allergens in children with and without peanut allergy, *Clin. Exp. Allergy* 40 (4) (2010) 590–597.
- [29] C. Radauer, M. Bublin, S. Wagner, A. Mari, H. Breiteneder, Allergens are distributed into few protein families and possess a restricted number of biochemical functions, *J. Allergy Clin. Immunol.* 121 (4) (2008) 847–852 (e7).
- [30] S.J. Maleki, S. Chung, E.T. Champagne, J. Raufman, The effects of roasting on the allergenic properties of peanut proteins, *J. Allergy Clin. Immunol.* 106 (4) (2000) 763–768.
- [31] K.A. Bloom, F.R. Huang, R. Bencharitwong, L. Bardina, A. Ross, H.A. Sampson, et al., Effect of heat treatment on milk and egg proteins allergenicity, *Pediatr. Allergy Immunol.* 25 (8) (2014) 740–746.
- [32] S.P. Commins, T.A. Platts-Mills, Delayed anaphylaxis to red meat in patients with IgE specific for galactose alpha-1, 3-galactose (alpha-gal), *Curr. Allergy Asthma Rep.* 13 (1) (2013) 72–77.
- [33] J.G. Michael, The role of digestive enzymes in orally induced immune tolerance, *Immunol. Invest.* 18 (9–10) (1989) 1049–1054.
- [34] S.H. Sicherer, A.W. Burks, H.A. Sampson, Clinical features of acute allergic reactions to peanut and tree nuts in children, *Pediatrics* 102 (1) (1998) e6–e6.
- [35] P. Van Asperen, A. Kemp, C. Mellis, Immediate food hypersensitivity reactions on the first known exposure to the food, *Arch. Dis. Child.* 58 (4) (1983) 253–256.
- [36] G. Lack, D. Fox, K. Northstone, J. Golding, Factors associated with the development of peanut allergy in childhood, *New Engl. J. Med.* 348 (11) (2003) 977–985.
- [37] N.P. Birmingham, S. Parvataneni, H.M.A. Hassan, J. Harkema, S. Samineni, L. Navuluri, et al., An adjuvant-free mouse model of tree nut allergy using hazelnut as a model tree nut, *Int. Arch. Allergy Immunol.* 144 (3) (2007) 203–210.
- [38] B. Gonipeta, S. Parvataneni, R. Tempelman, V. Gangur, An adjuvant-free mouse model to evaluate the allergenicity of milk whey protein, *J. Dairy Sci.* 92 (10) (2009) 4738–4744.
- [39] S. Parvataneni, B. Gonipeta, R.J. Tempelman, V. Gangur, Development of an adjuvant-free cashew nut allergy mouse model, *Int. Arch. Allergy Immunol.* 149 (4) (2009) 299–304.
- [40] M.K. Oyoshi, R.P. Larson, S.F. Ziegler, R.S. Geha, Mechanical injury polarizes skin dendritic cells to elicit a T H 2 response by inducing cutaneous thymic stromal lymphopoietin expression, *J. Allergy Clin. Immunol.* 126 (5) (2010) 976–984 (e5).
- [41] W. Li, Z. Zhang, A. Saxon, K. Zhang, Prevention of oral food allergy sensitization via skin application of food allergen in a mouse model, *Allergy* 67 (5) (2012) 622–629.
- [42] L. Mondoulet, V. Dioszeghy, E. Puteaux, M. Ligouis, V. Dhelft, F. Letourneur, et al., Intact skin and not stripped skin is crucial for the safety and efficacy of peanut epicutaneous immunotherapy (EPIT) in mice, *Clin. Transl. Allergy* 2 (1) (2012) 1.
- [43] G. Lack, Update on risk factors for food allergy, *J. Allergy Clin. Immunol.* 129 (5) (2012) 1187–1197.
- [44] S.H. Sicherer, R.A. Wood, D. Stablein, R. Lindblad, A.W. Burks, A.H. Liu, et al., Maternal consumption of peanut during pregnancy is associated with peanut sensitization in atopic infants, *J. Allergy Clin. Immunol.* 126 (6) (2010) 1191–1197.
- [45] K.M. Järvinen, J. Westfall, M. De Jesus, N.J. Mantis, J.A. Carroll, D.W. Metzger, et al., Role of maternal dietary peanut exposure in development of food allergy and oral tolerance, *PLoS One* 10 (12) (2015) e0143855.
- [46] A.E. Hoyt, T. Medico, S.P. Commins, Breast milk and food allergy: connections and current recommendations, *Pediatr. Clin. North Am.* 62 (6) (2015) 1493–1507.
- [47] T. Yamamoto, Y. Tsubota, T. Kodama, N. Kageyama-Yahara, M. Kadowaki, Oral tolerance induced by transfer of food antigens via breast milk of allergic mothers prevents offspring from developing allergic symptoms in a mouse food allergy model, *Clin. Dev. Immunol.* 2012 (2012).
- [48] D.P. Snider, J.S. Marshall, M.H. Perdue, H. Liang, Production of IgE antibody and allergic sensitization of intestinal and peripheral tissues after oral immunization with protein Ag and cholera toxin, *J. Immunol.* 153 (2) (1994) 647–657.
- [49] N. Foss, M. Duranti, C. Magni, H. Frøkiær, Assessment of lupin allergenicity in the cholera toxin model: induction of IgE response depends on the intrinsic properties of the conglutins and matrix effects, *Int. Arch. Allergy Immunol.* 141 (2) (2006) 141–150.
- [50] F. Anjuère, C. Luci, M. Lebens, D. Rousseau, C. Hervouet, G. Milon, et al., In vivo adjuvant-induced mobilization and maturation of gut dendritic cells after oral administration of cholera toxin, *J. Immunol.* 173 (8) (2004) 5103–5111.
- [51] M.C. Gagliardi, F. Sallusto, M. Marinaro, A. Langenkamp, A. Lanzavecchia, M.T. De Magistris, Cholera toxin induces maturation of human dendritic cells and licenses them for Th2 priming, *Eur. J. Immunol.* 30 (8) (2000) 2394–2403.
- [52] N. Anosova, S. Chabot, V. Shreedhar, J. Borawski, B. Dickinson, M. Neutra, Cholera toxin, E. coli heat-labile toxin, and non-toxic derivatives induce dendritic cell migration into the follicle-associated epithelium of Peyer's patches, *Mucosal Immunol.* 1 (1) (2008) 59–67.
- [53] J. Smit, M. Bol-Schoenmakers, I. Hassing, D. Fiechter, L. Boon, R. Bleumink, et al., The role of intestinal dendritic cells subsets in the establishment of food allergy, *Clin. Exp. Allergy* 41 (6) (2011) 890–898.
- [54] Ana Belén Blázquez, M. Cecilia Berin, Gastrointestinal dendritic cells promote Th2 skewing via OX40L, *J. Immunol.* 180 (7) (2008) 4441–4450.
- [55] Anna Stern, Agnes E. Wold, Sofia Östman, Neonatal mucosal immune stimulation by microbial superantigen improves the tolerogenic capacity of CD103+ dendritic cells, *PLoS One* 8 (9) (2013) e75594.
- [56] Ganesan Kirthana, et al., Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model, *J. Allergy Clin. Immunol.* 123 (1) (2009) 231–238.e4 (e4).
- [57] M. Cecilia Berin, Mechanisms of allergic sensitization to foods: bypassing immune tolerance pathways, *Immunol. Allergy Clin. North Am.* 32 (1) (2012) 1–10.
- [58] June L. Round, Sarkis K. Mazmanian, Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota, *Proc. Natl. Acad. Sci.* 107 (27) (2010) 12204–12209.
- [59] Koji Atarashi, et al., Induction of colonic regulatory T cells by indigenous Clostridium species, *Science* 331 (6015) (2011) 337–341.
- [60] Bertrand Rodriguez, et al., Infant gut microbiota is protective against cow's milk allergy in mice despite immature ileal T-cell response, *FEMS Microbiol. Ecol.* 79 (1) (2012) 192–202.
- [61] Bertrand Rodriguez, 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.* 76 (1) (2011) 133–144.
- [62] Magali Noval Rivas, et al., A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis, *J. Allergy Clin. Immunol.* 131 (1) (2013) 201–212.
- [63] Thomas R. Abrahamsson, et al., Low diversity of the gut microbiota in infants with atopic eczema, *J. Allergy Clin. Immunol.* 129 (2) (2012) 434–440 (e2).
- [64] Margherita Di Costanzo, Antonio Amoroso, Roberto Berni Canani, Gut microbiota as a target for food allergy, *J. Pediatr. Gastroenterol. Nutr.* 63 (1S) (2016) S11–S13.
- [65] Merja Nermes, Seppo Salminen, Erika Isolauri, Is there a role for probiotics in the prevention or treatment of food allergy? *Curr. Allergy Asthma Rep.* 13 (6) (2013) 622–630.
- [66] Mimi L.K. Tang, et al., Administration of a probiotic with peanut oral immunotherapy: a randomized trial, *J. Allergy Clin. Immunol.* 135 (3) (2015) 737–744 (e8).
- [67] Swantje I. Hammerschmidt, et al., Stromal mesenteric lymph node cells are essential for the generation of gut-homing T cells in vivo, *J. Exp. Med.* 205 (11) (2008) 2483–2490.
- [68] M. Iwata, et al., Retinoic acid imprints gut-homing specificity on T cells, *Immunity* 21 (4) (2004) 527–538.
- [69] Rosita Zakariaeabkoo, et al., Are vitamins A and D important in the development of food allergy and how are they best measured? *Clin. Biochem.* 47 (9) (2014) 804–811.
- [70] Jae-Hoon Chang, et al., 1, 25-Dihydroxyvitamin D 3 inhibits the differentiation

- and migration of Th 17 cells to protect against experimental autoimmune encephalomyelitis, *PLoS One* 5 (9) (2010) e12925.
- [71] Sanhong Yu, et al., Failure of T cell homing, reduced CD4/CD8 α intraepithelial lymphocytes, and inflammation in the gut of vitamin D receptor KO mice, *Proc. Natl. Acad. Sci.* 105 (52) (2008) 20834–20839.
- [72] Danny Bruce, Margherita T Cantorna, Intrinsic requirement for the vitamin D receptor in the development of CD8 α -expressing T cells, *J. Immunol.* 186 (5) (2011) 2819–2825.
- [73] Nicholas J. Osborne, et al., Prevalence of eczema and food allergy is associated with latitude in Australia, *J. Allergy Clin. Immunol.* 129 (3) (2012) 865–867.
- [74] Shimi Sharief, et al., Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005–2006, *J. Allergy Clin. Immunol.* 127 (5) (2011) 1195–1202.
- [75] Joshua D. Milner, et al., Early infant multivitamin supplementation is associated with increased risk for food allergy and asthma, *Pediatrics* 114 (1) (2004) 27–32.
- [76] Susan A Rudders, Carlos A Camargo Jr., Sunlight, vitamin D and food allergy, *Curr. Opin. Allergy Clin. Immunol.* 15 (4) (2015) 350–357.
- [77] Noor H.A. Suaini, et al., Immune modulation by vitamin D and its relevance to food allergy, *Nutrients* 7 (8) (2015) 6088–6108.
- [78] Robert A. Wood, et al., The natural history of milk allergy in an observational cohort, *J. Allergy Clin. Immunol.* 131 (3) (2013) 805–812 (e4).
- [79] Scott H. Sicherer, et al., The natural history of egg allergy in an observational cohort, *J. Allergy Clin. Immunol.* 133 (2) (2014) 492–499 (e8).
- [80] Ruchi S. Gupta, et al., Factors associated with reported food allergy tolerance among US children, *Ann. Allergy Asthma Immunol.* 111 (3) (2013) 194–198 (e4).
- [81] David M. Fleischer, The natural history of peanut and tree nut allergy, *Curr. Allergy Asthma Rep.* 7 (3) (2007) 175–181.
- [82] Helen S. Skolnick, et al., The natural history of peanut allergy, *J. Allergy Clin. Immunol.* 107 (2) (2001) 367–374.
- [83] Rachel L. Peters, et al., Natural history of peanut allergy and predictors of resolution in the first 4 years of life: a population-based assessment, *J. Allergy Clin. Immunol.* 135 (5) (2015) 1257–1266 (e2).
- [84] Sirasuda Sommanus, et al., Cow's milk protein allergy: immunological response in children with cow's milk protein tolerance, *Asian Pac. J. Allergy Immunol.* 32 (2) (2014) 171.
- [85] Emma M. Savilahti, et al., Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes, *J. Allergy Clin. Immunol.* 125 (6) (2010) 1315–1321 (e9).
- [86] M. Vazquez-Ortiz, et al., Serum allergen-specific IgA is not associated with natural or induced tolerance to egg in children, *Allergy* 68 (10) (2013) 1327–1332.
- [87] N. Qamar, et al., Naturally occurring tolerance acquisition to foods in previously allergic children is characterized by antigen specificity and associated with increased subsets of regulatory T cells, *Clin. Exp. Allergy* 45 (11) (2015) 1663–1672.
- [88] A Wesley Burks, et al., Oral immunotherapy for treatment of egg allergy in children, *New Engl. J. Med.* 367 (3) (2012) 233–243.
- [89] Stacie M. Jones, et al., Clinical efficacy and immune regulation with peanut oral immunotherapy, *J. Allergy Clin. Immunol.* 124 (2) (2009) 292–300 (e97).
- [90] Betty C.A.M. van Esch, et al., Oral tolerance induction by partially hydrolyzed whey protein in mice is associated with enhanced numbers of Foxp3+ regulatory T-cells in the mesenteric lymph nodes, *Pediatr. Allergy Immunol.* 22 (8) (2011) 820–826.
- [91] Aleena Syed, 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.* 133 (2) (2014) 500–510 (e11).
- [92] Brian P. Vickery, et al., Peanut oral immunotherapy modifies IgE and IgG 4 responses to major peanut allergens, *J. Allergy Clin. Immunol.* 131 (1) (2013) 128–134 (e3).
- [93] Gorelik Mark, et al., Suppression of the immunologic response to peanut during immunotherapy is often transient, *J. Allergy Clin. Immunol.* 135 (5) (2015) 1283–1292.
- [94] Rafael Pires Oliveira, et al., Antigen administration by continuous feeding enhances oral tolerance and leads to long-lasting effects, *J. Immunol. Methods* 421 (2015) 36–43.
- [95] Harold S. Nelson, et al., Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract, *J. Allergy Clin. Immunol.* 99 (6) (1997) 744–751.
- [96] Stacie M. Jones, et al., Safety of epicutaneous immunotherapy for the treatment of peanut allergy: a phase 1 study using the Viaskin patch, *J. Allergy Clin. Immunol.* 137 (4) (2016) 1258.
- [97] Vincent Dioszeghy, et al., Differences in phenotype, homing properties and suppressive activities of regulatory T cells induced by epicutaneous, oral or sublingual immunotherapy in mice sensitized to peanut *Cellular & Molecular Immunology* advance online publication 11 April 2016; doi: 10.1038/cmi.2016.14
- [98] Stacy J. Chin, et al., Sublingual versus oral immunotherapy for peanut-allergic children: a retrospective comparison, *J. Allergy Clin. Immunol.* 132 (2) (2013) 476.
- [99] Allison J. Burbank, et al., Oral immunotherapy for food allergy, *Immunol. Allergy Clin. North Am.* 36 (1) (2016) 55–69.
- [100] K. Blumchen, H. Ulbricht, U. Staden, K. Dobberstein, J. Beschoner, L. de Oliveira, et al., Oral peanut immunotherapy in children with peanut anaphylaxis, *Journal of Allergy and Clinical Immunology* 126 (2010) 83–91 e1.
- [101] P. Varshney, S. Jones, A. Scurlock, T. Perry, A. Kemper, P. Steele, et al., A randomized controlled study of peanut oral immunotherapy: Clinical desensitization and modulation of the allergic response, *Journal of Allergy and Clinical Immunology* 127 (2011) 654–660.
- [102] K. Anagnostou, S. Islam, Y. King, L. Foley, L. Pasea, C. Palmer, et al., Study of induction of Tolerance to Oral Peanut: a randomised controlled trial of desensitisation using peanut oral immunotherapy in children (STOP II), *Efficacy and Mechanism Evaluation* . 1 (2014) 1–56.
- [103] B. Vickery, A. Scurlock, M. Kulis, P. Steele, J. Kamilaris, J. Berglund, et al., Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy, *Journal of Allergy and Clinical Immunology* 133 (2014) 468–475 e6.
- [104] A. Buchanan, T. Green, S. Jones, A. Scurlock, L. Christie, K. Althage, et al., Egg oral immunotherapy in nonanaphylactic children with egg allergy, *Journal of Allergy and Clinical Immunology* 119 (2007) 199–205.
- [105] L. Caminiti, G. Pajno, G. Crisafulli, F. Chiera, M. Collura, G. Panasci, et al., Oral Immunotherapy for Egg Allergy: A Double-Blind Placebo-Controlled Study, with Postdesensitization Follow-Up, *The Journal of Allergy and Clinical Immunology* 3 (2015) 532–539 In Practice.
- [106] G. Longo, E. Barbi, I. Berti, R. Meneghetti, A. Pittalis, L. Ronfani, et al., Specific oral tolerance induction in children with very severe cow's milk-induced reactions, *Journal of Allergy and Clinical Immunology* 121 (2008) 343–347.
- [107] J. Skripak, S. Nash, H. Rowley, N. Brereton, S. Oh, R. Hamilton, et al., A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy, *Journal of Allergy and Clinical Immunology* 122 (2008) 1154–1160.
- [108] S. Narisety, P. Frischmeyer-Guerrero, C. Keet, M. Gorelik, J. Schroeder, R. Hamilton, et al., A randomized, double-blind, placebo-controlled pilot study of sublingual versus oral immunotherapy for the treatment of peanut allergy, *Journal of Allergy and Clinical Immunology* 135 (2015) 1275–1282 e6.
- [109] C. Keet, P. Frischmeyer-Guerrero, A. Thyagarajan, J. Schroeder, R. Hamilton, S. Boden, et al., The safety and efficacy of sublingual and oral immunotherapy for milk allergy, *Journal of Allergy and Clinical Immunology* 129 (2012) 448–455 e5.
- [110] D. Fleischer, A. Burks, B. Vickery, A. Scurlock, R. Wood, S. Jones, et al., Sublingual immunotherapy for peanut allergy: A randomized, double-blind, placebo-controlled multicenter trial, *Journal of Allergy and Clinical Immunology* 131 (2013) 119–127 e7.
- [111] A. Burks, R. Wood, S. Jones, S. Sicherer, D. Fleischer, A. Scurlock, et al., Sublingual immunotherapy for peanut allergy: Long-term follow-up of a randomized multicenter trial, *Journal of Allergy and Clinical Immunology* 135 (2015) 1240–1248 e3.