

Oral tolerance and oral immunotherapy for food allergy: Evidence for common mechanisms?

Wajiha Kazmi, M. Cecilia Berin^{*}

Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

ABSTRACT

Food allergies affect up to 10% of the US population, can be life-threatening, and have a significant negative impact on quality of life. Delayed dietary introduction of foods in childhood can hinder the induction of oral tolerance, an active regulatory response to foods that prevents the development of food allergy. Some children outgrow their food allergies naturally, while many others have persistent, lifelong food allergy for which there are few therapeutic options. Oral immunotherapy (OIT) is a therapeutic approach of giving increasing amounts of food to attempt to desensitize the allergic individual. In this review, we focus on the immune mechanisms common to oral tolerance and response to oral immunotherapy, with the objective of determining whether true tolerance can be achieved after food allergy has been established.

1. Introduction

Food allergies have been increasing at a rapid rate over the past decade and prevalence of food allergy has been reported to be as high as 10 % in the United States [1], representing a significant public health concern. There is currently no cure for food allergy, and management or treatment options include avoidance of the allergic food, desensitization through oral immunotherapy (OIT), or self-injection of epinephrine if anaphylaxis occurs due to an accidental exposure to the allergen [2]. Peanut allergy, one of the most common food allergies in the US, is often severe and life-long, accounting for the majority of cases of anaphylaxis in all ages.

Food allergy arises as a result of the inappropriate immune reactivity to food antigens. Food allergies can be IgE-mediated, non-IgE-mediated, or disorders with mixed IgE-mediated and cell-mediated immune reactions. Oral tolerance is a state of systemic unresponsiveness or hyporesponsiveness that occurs in response to food antigens and is the default physiological immune response to food antigens. In subjects with food allergy, tolerance to food antigens can arise naturally if they outgrow the allergy, termed natural resolution. In the clinical setting, tolerance to allergenic foods is aimed to be induced through oral immunotherapy (OIT), with or without adjunct therapy. Presently, oral immunotherapy has been successful in inducing desensitization to food antigens, which is a transient reduced clinical reactivity. OIT has also been able to induce sustained clinical benefit, described as remission or sustained unresponsiveness, terms that have been used to describe individuals who can pass an oral food challenge (OFC) after stopping OIT, demonstrating

relative protection from clinical allergic reactions [3]. The longer the period of avoidance, the fewer individuals maintain protection [4], suggesting a lack of true tolerance. Gaining a better understanding of oral tolerance and its mechanisms is crucial for the development of novel prevention and treatment strategies for food allergies.

This review provides an overview of currently known mechanisms of oral tolerance in IgE-mediated food allergy from animal models and human in vitro assay studies. Next, we discuss known mechanisms of oral immunotherapy and natural resolution, which is achieved when a child outgrows their food allergy. Lastly, we discuss the differences between oral tolerance that is achieved through early exposure to allergen and that which is aimed to be achieved through oral immunotherapy, further examining whether oral immunotherapy induces desensitization or true immune tolerance. We conclude with a consideration of future directions of oral immunotherapy.

2. Oral tolerance in murine models

Oral tolerance is a concept derived from experimental models. Oral exposure to an antigen renders the animal hyporesponsive to that antigen upon subsequent immunization. When Wells and Osborne first described oral tolerance, they reported that guinea pigs could not be induced to undergo anaphylaxis to antigens that were already present in their diet [5]. This phenomenon is thought to be the underlying mechanism by which organisms tolerate the large quantities of foreign proteins they ingest daily.

From murine models of food allergy, it has been demonstrated that

^{*} Corresponding author.

E-mail address: cecilia.berin@northwestern.edu (M.C. Berin).

oral tolerance occurs through the following major mechanisms: T cell anergy and depletion, the induction of antigen-specific Tregs, and the production of IgG. Classically, exposure to high levels of food antigen has been shown to induce T cell anergy and depletion [6], while repeated low levels of antigen exposure induces regulatory T cells. However, evidence suggests they are not mutually exclusive [6]. Anergic T cells, despite an ability to bind antigen presenting cells, lack the ability to elicit immune responses. Upregulation of genes involved in T cell anergy is mediated by nuclear factor of activated T cells (NFAT) [7]. Further, effector T cells have been depleted upon high antigen exposure, contributing to oral tolerance.

A role for induced Tregs in promoting oral tolerance was shown with depletion of regulatory T cell (DEREG) mice that have the diphtheria toxin receptor driven by the Foxp3 promoter, resulting in selective depletion of Tregs after injection of diphtheria toxin. Depletion of Tregs after oral antigen exposure, followed by a delay to allow Tregs to repopulate before immunizing abolished oral tolerance in murine models, demonstrating the role of antigen-specific Tregs in oral tolerance and prevention of experimental food allergy [8]. This is consistent with earlier work that identified induced Tregs, rather than thymic Tregs, in the development of oral tolerance [9]. Further, Turner et al identified Treg-derived TGF- β 1 as playing a major role in the prevention of food allergy, as a reduction in Treg-produced TGF- β 1 through haploinsufficiency was sufficient to lead to food allergy while a complete loss of Treg-produced TGF- β 1 led to autoimmunity [10]. Non-canonical T cells have also been identified to play a role in the development of oral tolerance. Hong et al recently showed that in addition to induction of Tregs, gliadin feeding induces a population of CD4 + T helper cells of undefined helper lineage with an anergic phenotype that are resistant to developing into effector T cells, but can develop into Tregs under the influence of IL-2 [11]. In addition to Tregs and anergic T cells, gliadin-feeding induced a small population of T follicular helper (Tfh) cells that supported a weak anti-gliadin IgG response.

IgG has received less recognition as an important mechanism of oral tolerance in mouse models. IgG in breastmilk was shown to be an important factor in the transfer of tolerance from mother to offspring [12,13]. In murine models of oral tolerance, IgG has been shown to prevent mast cell degranulation by steric blockade and by crosslinking of cellular Fc receptors [14]. Burton et al showed that administration of allergen-specific IgG to allergy-prone mice before sensitization led to a significant reduction of food allergy through preventing IgE antibody development, and this was dependent on the interaction of IgG with inhibitory IgG receptor Fc γ RIIb on mast cells. Additionally, IgG administration in mice with established IgE-mediated food allergy led to Treg expansion and suppression of the Th2 response [15]. Under typical oral tolerance model conditions, where mice are fed with antigen for a short period of time (i.e. a week) before immunization, it is unlikely that IgG would play a significant role. However, in the context of chronic antigen exposure that mimics human dietary exposure, IgG antibodies may play a more critical role.

3. Oral tolerance and prevention of food allergy in clinical trials

Food allergy is often cited as a failure of oral tolerance, but reactions to foods such as peanuts and tree nuts most often occur on the first known ingestion of the food [16]. A growing body of evidence indicates that the site of exposure leading to allergic sensitization in early life is actually the skin, at least for allergens such as peanut [17]. Therefore, it was unclear if food allergy is a failure of oral tolerance, or a lack of opportunity to develop oral tolerance prior to the onset of sensitization. It was noted that rates of peanut allergy were much lower in Israel than among Jewish schoolchildren in London, and this lower prevalence was associated with common introduction of peanut into the diet in the first year of life [18]. To directly address whether early introduction of peanut protected from peanut allergy, the Learning Early about Peanuts (LEAP) study was a prospective, randomized clinical trial that enrolled

640 infants with severe eczema, egg allergy, or both who were at high risk of developing peanut allergy [19]. This landmark clinical trial was the first to demonstrate that early introduction of peanut protein to the diet decreased the incidence of peanut allergy in children at 60 months of age by 81 % compared with infants who avoided oral ingestion of peanuts. To determine if the protection from peanut allergy was true tolerance, the Persistence of Oral Tolerance to Peanut (LEAP-ON) study evaluated the persistence of protection from peanut allergy in LEAP participants who avoided peanut for a full 12 months [20]. Results showed that after 12 months of peanut avoidance, only 4.8 % of the original peanut consumers were found to be allergic, compared to 18.6 % of the original peanut avoiders, a highly significant difference. The induction of oral tolerance was allergen specific, as no impact of early peanut ingestion was found on development of egg allergy [21]. This shows that early introduction of allergens is highly effective at preventing the development of peanut allergy, even in children with a high risk of developing peanut allergy. Therefore, food allergy appears to be a result of a missed opportunity to establish oral tolerance rather than non-functional tolerance mechanisms.

A number of other studies have examined the impact of early food introduction on preventing food allergy. The Enquiring About Tolerance (EAT) study explored the broad applicability of the LEAP study by investigating early introduction of six allergenic foods (peanut, egg, milk, sesame, fish, wheat) at the age of three months along with breastfeeding compared to exclusive breastfeeding in a cohort of infants in the UK without increased risk of food allergy [22]. Although early introduction of multiple foods was found to be challenging, it was determined to be safe and intention to treat analysis confirmed a protective effect of early introduction of peanut and egg, with significant reduction of peanut and egg allergy prevalence in the early introduction group.

Mechanisms of tolerance in the LEAP study have primarily focused on humoral immune responses. Although peanut-specific (ps) IgE levels rose in both consumption and avoidance groups, fewer individuals developed very high ps-IgE in the consumption group [19]. Levels of ps-IgG4 rose earlier and to a greater extent in the consumption group than the avoidance group. Suarez-Farinas et al examined the epitope-specific IgE and IgG4 response over time in the LEAP cohort [23]. Only those in the avoidance group developed significant levels of epitope-specific IgE, with early responses being restricted to whole proteins indicating conformational epitopes. In the consumption group, the epitope-specific IgG4 response was greater in magnitude and earlier than the avoidance group, as was the ps-IgG4 response. Presence of IgG antibodies have been shown to promote the development of oral tolerance in mouse models [15].

Regulation of the humoral immune response at the cellular level has not yet been examined in the LEAP cohort. Genetic associations with susceptibility to developing food allergy are beginning to be characterized. In the LEAP cohort, sensitization in the peanut avoidance group was shown to be significantly associated with the mucosa-associated lymphoid tissue lymphoma translocation (MALT1) gene [24], which contributes to TCR signaling and is associated with the breadth of the peanut-specific IgE response. Carriers of the MALT1 variant were equally amenable to tolerance induction in the consumption group as non-carriers, indicating that this susceptibility to sensitization is not due to a defect in tolerance pathways. There are also genetic contributions to the IgG4 response to peanut, specifically the association of HLA alleles with the breadth of the IgG4 response to Ara h 2 that occurs with continued exposure to peanut protein [25]. The allele DQA1*01:02 that has previously been found to be associated with peanut allergy was also associated with the IgG4 response to peanut ingestion, indicating that it is likely permissive to binding of immunogenic peanut epitopes.

In the absence of cellular studies from the LEAP cohort, a mouse model was developed to mimic the LEAP study [26]. Kremeski et al showed that after intranasal or epicutaneous sensitization of mice to peanut antigen, naïve mice developed peanut allergy, while those who

had oral ingestion of peanut butter prior to sensitization effectively prevented the development of peanut allergy. This was due to regulation of the Tfh response by Tregs expressing elevated levels of Cytotoxic T-lymphocyte associated protein 4 (CTLA4), and blocking CTLA4 abolished the protective effect of oral peanut. The evolution of the peanut specific T cell response in early life, including Tfh cells and Treg cells, is poorly understood in humans and awaits study. However, it is known that loss of Tregs associated with Foxp3 mutations is associated with severe food allergy [27], underscoring the important role of Tregs in maintaining tolerance to foods.

4. Natural resolution of food allergy

Most children outgrow their milk or egg allergies, while allergies to peanut, tree nuts, fish and shellfish are more commonly lifelong. In a cohort of allergic children enrolled between 3 and 15 months, 50 % of children outgrew their milk allergy by 5 years of age [28], and 50 % outgrew their egg allergy by 6 years of age [29]. In the HealthNuts cohort based in Australia, 89 % of children outgrew their egg allergy by age 6 compared to 29 % who outgrew their peanut allergy [30]. Natural resolution through outgrowing food allergy is considered true tolerance due to rarity of allergy recurrence, but there have been few studies on the mechanisms underlying allergy resolution. It is also not understood why some food allergies are more readily outgrown, but this may relate to the intrinsic adjuvant activity of foods such as peanut [31].

Tregs and inhibitory cytokines have been shown to mediate the resolution of food allergy. Development of tolerance to milk in the context of non-IgE-mediated food allergy is associated with the expansion of circulating CD4 + CD25 + regulatory T cells [32]. Children who outgrew their allergy were shown to have higher numbers of circulating CD4 + CD25 + T cells and decreased *in vitro* proliferative responses to bovine β -lactoglobulin in peripheral blood mononuclear cells (PBMCs) than children who had clinically active allergy. Furthermore, CD25 + cell depletion in PBMCs of tolerant children lead to a 5-fold increase in *in vitro* proliferation against β -lactoglobulin. Individuals who had naturally outgrown their egg allergy had lower levels of Th2 cytokines and higher levels of inhibitory cytokines IL-10 and IFN- γ from egg-restimulated PBMCs than allergic controls [33]. Qamar et al found that resting Tregs (CD3 + CD4 + Foxp3 + CD25 + CD127lo) were increased in those with recently outgrown peanut or egg allergy and that IL-10 production from total CD4 + T cells and Tregs was increased in response to allergen stimulation [34].

Studies have also focused on identifying risk factors associated with persistence of food allergy. While children often outgrow milk and egg allergies, allergies to peanuts and tree nuts are often persistent. Persistence of peanut allergy was associated with having early onset severe eczema at 1 year of age, sensitization to other foods, and having a lower symptom-eliciting dose during an oral food challenge [35,30]. In the context of egg allergy, having a more diverse anti-egg IgE repertoire (recognition of multiple egg allergens) was associated with persistence [36]. Neeland et al examined the role of innate immunity in egg allergy persistence and found an increased pro-inflammatory response to lipopolysaccharide (LPS) stimulation in those with persistent disease [35].

Epigenetic changes have been described in children who had persistence of egg allergy compared to those who had resolution [37]. Methylation profiling analyses have highlighted the epigenetic modification of T cell activation pathways in children with egg allergy [38]. In children who failed to resolve egg allergy in later childhood, there were increases in epigenetic disruption at T cell activation genes and poor lymphoproliferative responses compared to children who had outgrown their food allergy [37]. Persistence of egg allergy was also associated with increased IFN- γ production, however Th2 cytokines were not reported so it is not known if the Th2/Th1 balance is altered in persistent egg allergy. In children with resolution of egg allergy, differences in DNA methylation were found to be stable in a core set of remodeled metabolic and inflammatory genes associated with food allergy [38].

More work is needed to study the T cell and B cell interactions involved in natural resolution of food allergy to determine how much of natural resolution is an active regulatory process similar to the generation of oral tolerance.

5. Oral immunotherapy

Individuals with persistent food allergy need therapeutic options, which are very limited at this time. Allergen oral immunotherapy (OIT) is a disease modifying treatment strategy for food allergic individuals that has been studied over the past two decades. During OIT, small amounts of allergen are given to a confirmed allergic individual over time until they are able to safely tolerate higher amounts of allergen without evidence of clinical symptoms, which is termed desensitization [2]. The term tolerance has not been used in the context of food allergy immunotherapy trials due to the difficulty in demonstrating the permanence of the clinical protection. Sustained unresponsiveness (SU) has been used to describe clinical non-reactivity while avoiding foods/therapy and is defined by passing an oral food challenge after periods of time ranging from 2 weeks up to 1 year of food avoidance following treatment. The term remission has recently been used in place of SU [3], which emphasizes the concept that the clinical benefit from OIT may not be permanent. In 2020, the oral drug Palforzia (Peanut (Arachis hypogaea) Allergen Powder- dnpf) containing standardized peanut protein gained approval by the US Food and Drug Administration as an OIT for the treatment of peanut allergy.

It has been reported through various OIT studies that approximately 80 % of participants have a successful response to OIT by achieving desensitization and of those approximately 30–50 % are able to achieve SU. Additionally, 10–15 % of participants do not achieve desensitization and may withdraw from OIT due to persistent dose-related adverse events such as gastrointestinal symptoms. Randomized controlled trials have indicated that intervention with OIT at earlier ages, ie age three and younger, has enhanced SU or remission outcomes and is able to disrupt peanut allergy with a shorter duration of treatment [3,39]. The choice of time interval with which to test SU is particularly important, as shown in the POISED study [4]. 35 % of those avoiding peanut 16 weeks after stopping OIT could pass a 4000 g challenge and could be classified as SU, compared to 13 % at one year after stopping OIT. The average age in the POISED trial was 11 years. In the IMPACT trial, which enrolled children age 12–48 months, the rate of SU/remission was 29 % at 12 weeks post-OIT (by per-protocol analysis). However, when broken down by age groups the rate of remission was 71 % in 12–24 months, 35 % in 24–36 months, and 19 % in 36–48 months. Limitations are the low number of individuals in the youngest age group (n = 7). This high rate of remission in the youngest group was proposed to be attributable to the lower average peanut specific IgE levels typically seen in young children and due to the plasticity of the immune response at younger ages. Here we use the term plasticity to refer to the capacity to restore the overall immune response to one of homeostasis, it is not used to refer to plasticity within cell types. No mechanisms have yet been attributed to the capacity of younger children to achieve remission.

Immune mechanisms of OIT continue to be investigated and characterized. Humoral immune responses to OIT include an initial rise in specific IgE followed by a decrease over time [40,41]. Specific IgG4 levels have been observed to rise over the course of OIT and IgA levels also increase in response to OIT [42,43]. Baseline values and changes in specific antibodies have been related to clinical outcomes. A low level of specific IgE at baseline before OIT has been shown to be predictive of SU or remission, however changes in IgE levels after treatment do not distinguish groups by outcome. In contrast, IgG4 at baseline is not informative of treatment outcome, but increases in IgG4 post-treatment are associated with SU. Mechanistically, in addition to functioning as a blocking antibody, IgG induced by OIT has been shown to suppress basophil activation through activity on Fc γ RIIb, which activates phosphatases that inhibit Fc ϵ RI signaling. IgA, and specifically the IgA2

isotype, is increased in OIT treatment responders compared to non-responders [43]. IgA2 is more predominant at mucosal sites than in serum and is more stable than IgA1 and may reflect a local IgA response in the gastrointestinal mucosa during OIT. It is not known if the function of IgA is primarily in preventing mucosal antigen uptake or if there is another mechanism that may contribute to protection.

Consistent with a drop in IgE, studies have demonstrated that OIT reduces the activation of basophils measured by basophil activation test (BAT) compared to placebo [44,45]. Furthermore, early changes in BAT are predictive of SU [46], despite the poor predictive value of IgE. This suggests that inhibition at the cellular level may be important for SU. IgG4 and Tregs may also contribute to this suppression of basophils independent of IgE, or the suppression may be intrinsic to the basophil. Although basophils have been suggested to contribute to peanut allergy in humans [47], these changes measured by BAT may also relate to changes at the level of tissue mast cells that play a key role in food allergy [48,49].

There has been considerable focus on understanding the response of T cells to OIT. Initial studies demonstrated a reduction in allergen-induced Th2 cytokine secretion during OIT [50,51] and this is consistent with studies showing a decrease in frequency of Type 2 T cell subsets post-OIT [52–54]. Using CD154-based approaches to detect allergen-specific T cells, a reduction in Type 2 T cell frequency did not discriminate between treatment outcomes, but a high frequency of these cells at baseline was predictive of treatment failure [54]. Monian et al used single cell RNA sequencing together with TCR analysis to track different T helper subsets over the course of OIT, and found that OIT was associated with a significant suppression of Th2A-like cells, which are terminally differentiated CD4⁺ T cells expressing CRTH2, CD49d, and CD161, but was not associated with a change in Tfh2 cells or Th2-like Tregs [55]. Interestingly, they found that the frequency of Th2A cells rebounded after OIT was terminated, indicating that the memory reservoir of these cells remained intact. Altogether, these studies support a mechanism of deletion such as was originally proposed for high dose oral tolerance. Studies that have specifically addressed the dose of antigen during OIT (high dose 3000 mg, low dose 300 mg) found no difference in underlying immune mechanisms [56], although these may both constitute “high dose”.

The contribution of Tregs to the response to OIT remains more controversial. Varshney et al reported an increase in Foxp3^{high} CD4⁺ CD25⁺ cells post-OIT [57]. A similar finding of increased frequency of global Tregs post-OIT only in individuals with SU was recently reported from individuals treated with egg OIT [58]. Further, using a combination of CFSE-dilution and CD154-based detection of antigen-specific T cells, Syed et al found an increase in frequency of Foxp3⁺ CD4⁺ T cells and a demethylation of the Foxp3 locus in those with SU compared to desensitized individuals [59]. To profile T cells before and after OIT, Ryan et al used Ara h 2 tetramers to select peanut-specific T cells and used a single cell PCR-based profiling approach to [60]. They found an overall expansion of peanut-specific T cells after OIT, and a marked expansion of cells with an anergic phenotype, but there was no clear expansion of regulatory T cells identified through this approach. Similarly, Monian et al. used a single cell RNAseq analysis approach with cells captured using either CD154 or CD137 surface expression, and although they identified multiple Treg phenotypes, these cells were not changed in frequency or phenotype after OIT [55]. Wang et al. performed single cell targeted RNAseq analysis of peanut-specific T cells captured with the use of Ara h 2 dextramers [61]. They found an early increase in a population of activated Th2 cells (expressing high levels of STATs), followed by an increase in TGF- β expression particularly in those with treatment success. There was no cluster clearly identified as regulatory in phenotype. Zhang et al. had the unique opportunity to profile cells in the gastrointestinal tract of 3 individuals pre and post OIT [62]. They sorted $\gamma\delta$ T cells, and identified regulatory transcripts IL-10 and IL-2RA (CD25) among the differentially expressed genes post-OIT. The TGF- β signaling pathway was also significantly enriched,

suggesting a regulatory mechanism at play. Overall, several studies have pointed to a role for regulatory pathways or Tregs in the response to OIT, yet a number of studies that selected antigen-specific T cells have failed to identify changes in Treg populations. Differences in findings likely relate to differences in methods to identify “antigen-specific” T cells. Only tetramer and dextramer selection can identify true antigen-specific cells without the issue of bystander activation, but they have their own limitations (MHC II restriction of use, and the profiling of only a few epitopes out of the wide range of epitopes that are functional in food allergies). This raises the question of true antigen specificity of cells with regulatory activity and the role of Tregs in response to OIT remains an area of active research. No T cell studies have been published yet from the LEAP or IMPACT studies that could help to elucidate how young children are more amenable to remission or tolerance than older children.

6. Oral tolerance vs oral immunotherapy: Similarities and mechanistic divergences

Key similarities we highlighted prior between oral tolerance and successful OIT outcomes include introduction of the allergen at a younger age, as demonstrated by clinical trials of early allergen introduction leading to oral tolerance and prevention of food allergy as well the demonstration that OIT is more effective in young infants ages 9–36 months than in pre-school children. This suggests that earlier introduction is beneficial as the capacity of the immune system to return to homeostasis may be lost with age although the underlying immune characteristics of this are poorly understood. The increased success of OIT in early life may also be correlated with lower IgE levels present in younger individuals, but exact mechanisms continue to be investigated in clinical trials. Mechanistically, oral tolerance and OIT demonstrate a role of Tregs and IL-10-producing T cells in preventing reactivity to antigen, as well as an important role for T cell anergy. Furthermore, in oral tolerance there is low but detectable antigen-specific IgG to food allergens which may be particularly important in those with susceptibility to food allergy and low levels of IgE, and in OIT there also is generation of IgG4 antibodies which block IgE binding.

A significant limitation of OIT is that subjects often experience loss of protective clinical effects. While some subjects experience longer periods of clinical protection after OIT, termed SU or remission, SU particularly in older adolescents and adults may be closer in immunologic character to desensitization than true tolerance, as SU is often transient in older individuals [4]. Desensitization is dependent upon continued exposure to OIT. This may be due the important role for anergy of Type 2 T cell responses, which may recover over time when antigen is no longer being administered.

7. Can tolerance be established therapeutically in the context of food allergy?

The immune pathways involved in oral tolerance, food allergy, OIT, and natural resolution are summarized in Fig. 1. A key question in the field of food allergy is whether true tolerance or resolution of food allergy can be induced by oral immunotherapy. The LEAP and LEAP-ON studies showed that oral exposure even in infants who already had low levels of IgE led to long-lasting tolerance [19,20]. The IMPACT study showed a high level of remission in those treated in the first year of life [3]. Age appears to be critical for oral induction of tolerance when sensitization has already occurred. Natural resolution, which is more common for egg and milk, involves not only a loss of allergic pathways, but also appears to involve Tregs. Oral tolerance and OIT clearly share a protective mechanism of production of specific IgG4. It appears that in the context of low levels of IgE to primarily conformational epitopes-reflective of the response in young children or in the allergic response to foods such as egg- OIT is capable of providing longer term clinical benefit through sustained unresponsiveness. It is not clear if an IgG4

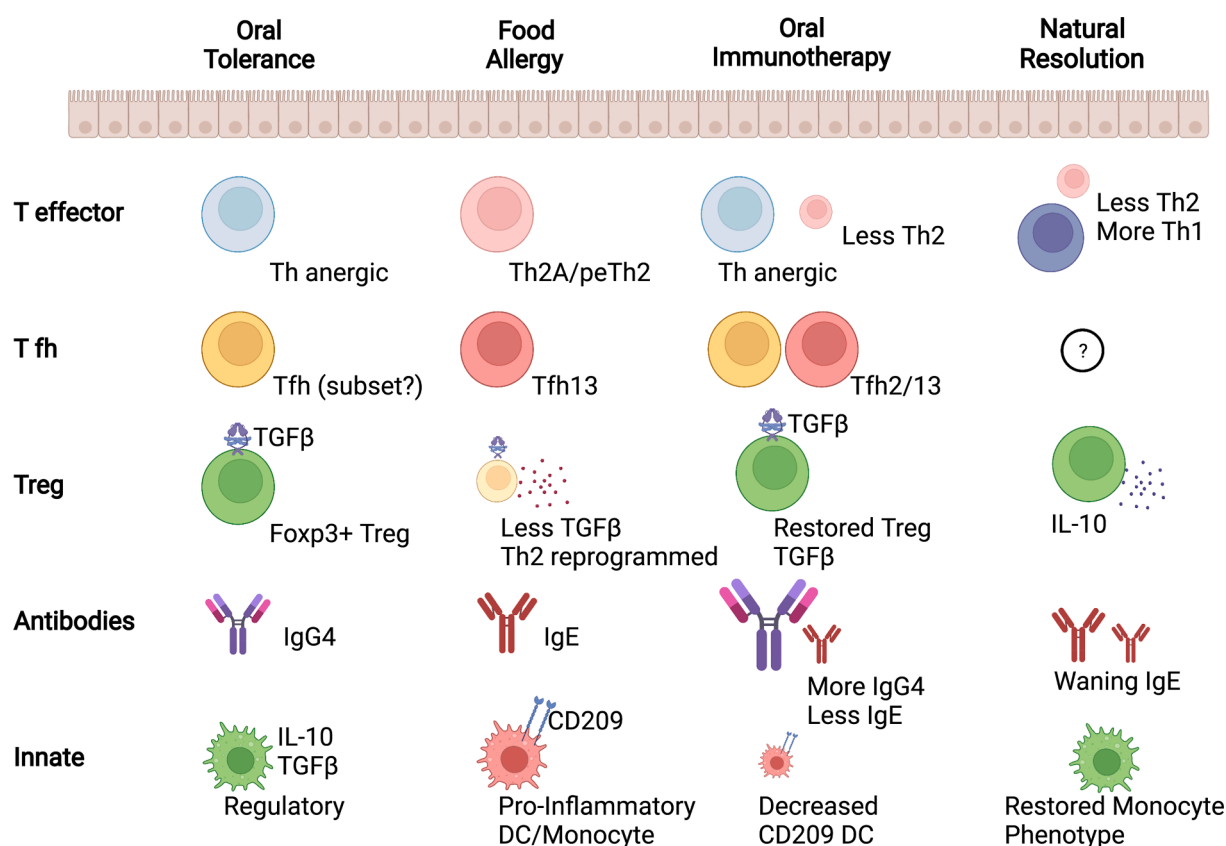


Fig. 1. Summary of Immune Features of Oral Tolerance, Food Allergy, Natural Resolution, and OIT. T cell responses are divided into CD4 + T effector cells, T follicular helper (Tfh), and regulatory (Treg) cell responses.

response is sufficient for protection in this context, or if Tregs have an important role. More data is needed on the evolution of the cellular (T cell and B cell) response to foods in early life in the context of allergy susceptibility and early intervention to understand the key barriers and mechanisms of successful OIT response.

8. Future directions of oral immunotherapy

There have been heterogeneous patient responses to OIT in clinical trials and there is a need to unravel the immune mechanisms that underpin successful response to OIT. Standardization of endpoints across future clinical trials could yield more information about which OIT approaches are clinically useful for achieving sustained unresponsiveness. Although some predictive biomarkers have been identified, there is a need for further characterization to predict which individuals will develop desensitization versus sustained unresponsiveness. There is also a need for identification of biomarkers that will predict adverse reactions to OIT, such as anaphylaxis and eosinophilic esophagitis.

Currently, the only widely-approved by regulatory agencies (i.e. FDA, NICE, EMA) OIT that exists is for peanut allergy, and there is a need for further research to develop standardized OIT for other major food allergens, particularly for those with a very low threshold of reactivity that cannot safely use measured quantities of foods. Further, combination therapy in OIT is an emerging area of interest, particularly since individuals with high IgE levels have the least benefit from OIT. Studies are investigating anti-IgE monoclonal antibody omalizumab as monotherapy and an adjunctive therapy for OIT and it has been shown to help reduce adverse effects and decrease time to achieve desensitization [63]. OIT for multiple food allergies using omalizumab induction treatment is currently being tested in a Phase 3 multicenter placebo-controlled clinical trial (NCT03881696). A key mechanism of treatment success appears to be the deletion or anergy of Type 2 CD4 + T cells. Biologics

that could enhance targeting of Type 2 immunity, such as JAK-inhibitors (NCT05069831) and antibodies targeting Th2 cytokines (NCT03682770) are under investigation and may help to bring OIT outcomes closer to true tolerance. Finally, there is pre-clinical evidence that CTLA-4 may contribute to immune tolerance to foods [26,64,65], and the CTLA-4 agonist abatacept is currently being tested in a clinical trial as an adjunct to OIT (NCT04872218).

Author statement

Wajiha Kazmi wrote the first draft of the manuscript, Cecilia Berin revised and finalized the manuscript for publication and prepared the figure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

- [1] J.P. Lopes, S. Sicherer, Food allergy: epidemiology, pathogenesis, diagnosis, prevention, and treatment, *Curr. Opin. Immunol.* 66 (2020) 57–64, <https://doi.org/10.1016/j.coi.2020.03.014>. PubMed PMID: 32446135. Epub 2020/05/24.
- [2] S.M. Jones, A.W. Burks, Food Allergy, *New Engl. J. Med.* 377 (12) (2017) 1168–1176, <https://doi.org/10.1056/NEJMcip1611971>. PubMed PMID: 28930512. Epub 2017/09/21.
- [3] S.M. Jones, E.H. Kim, K.C. Nadeau, A. Nowak-Wegrzyn, R.A. Wood, H.A. Sampson, A.M. Scurlock, S. Chinthrajah, J. Wang, R.D. Pesek, S.B. Sindher, M. Kulis,

- J. Johnson, K. Spain, D.C. Babineau, H. Chin, J. Laurienzo-Panza, R. Yan, D. Larson, T. Qin, D. Whitehouse, M.L. Sever, S. Sanda, M. Plaut, L.M. Wheatley, A. W. Burks, Tolerance N. Immune, Efficacy and safety of oral immunotherapy in children aged 1–3 years with peanut allergy (the Immune Tolerance Network IMPACT trial): a randomised placebo-controlled study, *Lancet* 399 (10322) (2022) 359–371, [https://doi.org/10.1016/S0140-6736\(21\)02390-4](https://doi.org/10.1016/S0140-6736(21)02390-4). PubMed PMID: 35065784; PMCID: PMC9119642. Epub 2022/01/24.
- [4] R.S. Chinthrajah, N. Purington, S. Andorf, A. Long, K.L. O’Laughlin, S.C. Lyu, M. Manohar, S.D. Boyd, R. Tibshirani, H. Maecker, M. Plaut, K. Mukai, M. Tsai, M. Desai, S.J. Galli, K.C. Nadeau, Sustained outcomes in oral immunotherapy for peanut allergy (POISED study): a large, randomised, double-blind, placebo-controlled, phase 2 study, *Lancet*. 394 (10207) (2019) 1437–1449, [https://doi.org/10.1016/S0140-6736\(19\)31793-3](https://doi.org/10.1016/S0140-6736(19)31793-3). PubMed PMID: 31522849. Epub 2019/09/17.
- [5] H.G. Wells, T.B. Osborne, The biological reactions of the vegetable proteins. I. Anaphylaxis, *J. Infect. Dis.* 8 (1911) 66–124.
- [6] 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. PubMed PMID: 7603570.
- [7] F. Macian, F. Garcia-Cozar, S.H. Im, H.F. Horton, M.C. Byrne, A. Rao, Transcriptional mechanisms underlying lymphocyte tolerance, *Cell* 109 (6) (2002) 719–731, [https://doi.org/10.1016/S0092-8674\(02\)00767-5](https://doi.org/10.1016/S0092-8674(02)00767-5). PubMed PMID: 12086671. Epub 2002/06/28.
- [8] U. Hadis, B. Wahl, O. Schulz, M. Hardtke-Wolenski, A. Schippers, N. Wagner, W. Muller, T. Sparwasser, R. Forster, O. Pabst, Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria, *Immunity* 34 (2) (2011) 237–246, <https://doi.org/10.1016/j.immuni.2011.01.016>. PubMed PMID: 21333554.
- [9] D. Mucida, N. Kutchukhidze, A. Erazo, M. Russo, J.J. Lafaille, M.A. Curotto de Lafaille, Oral tolerance in the absence of naturally occurring Tregs, *J. Clin. Invest.* 115 (7) (2005) 1923–1933. PubMed PMID: 15937545.
- [10] J.A. Turner, E. Stephen-Victor, S. Wang, M.N. Rivas, A. Abdel-Gadir, H. Harb, Y. Cui, M. Fanny, L.M. Charbonnier, J.J.H. Fong, M. Benamar, L. Wang, O. T. Burton, K. Bansal, L. Bry, C. Zhu, Q.Z. Li, R.L. Clement, H.C. Oettgen, E. Crestani, R. Rachid, P.T. Sage, T.A. Chatila, Regulatory T Cell-Derived TGF-beta1 Controls Multiple Checkpoints Governing Allergy and Autoimmunity, *Immunity*. 53 (6) (2020) 1202–14 e6, <https://doi.org/10.1016/j.immuni.2020.10.002>. PubMed PMID: 33086036; PMCID: PMC7744401. Epub 2020/10/22.
- [11] S.W. Hong, P.D. Krueger, K.C. Osum, T. Dileepan, A. Herman, D.L. Mueller, M. K. Jenkins, Immune tolerance of food is mediated by layers of CD4(+) T cell dysfunction, *Nature*. (2022), <https://doi.org/10.1038/s41586-022-04916-6>. PubMed PMID: 35794484. Epub 2022/07/07.
- [12] E. Mosconi, A. Rekima, B. Seitz-Polski, A. Kanda, S. Fleury, E. Tissandier, R. Monteiro, D.D. Dombrowicz, V. Julia, N. Glaichenhaus, V. Verhasselt, Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development, *Mucosal Immunology*. 3 (5) (2010) 461–474, <https://doi.org/10.1038/mi.2010.23> PubMed PMID: 20485331. Epub 2010/05/21.
- [13] A. Ohsaki, N. Venturielli, T.M. Buccigrosso, S.K. Osganian, J. Lee, R.S. Blumberg, M. K. Oyoshi, Maternal IgG immune complexes induce food allergen-specific tolerance in offspring, *J. Exp. Med.* 218:215(1):91–1Epub 2017/11/22. doi: 10.1084/jem.20171163. PubMed PMID: 29158374; PMCID: PMC5748859.
- [14] R.T. Strait, S.C. Morris, F.D. Finkelman, IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIB cross-linking, *J. Clin. Invest.* 116 (3) (2006) 833–841. PubMed PMID: 16498503.
- [15] O.T. Burton, J.M. Tamayo, A.J. Stranks, K.J. Koleoglou, H.C. Oettgen, Allergen-specific IgG antibody signaling through Fc gammaRIIB promotes food tolerance, *J. Allergy Clin. Immunol.* 141 (1) (2018) 189–201 e3, <https://doi.org/10.1016/j.jaci.2017.03.045>. PubMed PMID: 28479335; PMCID: PMC5671359. Epub 2017/05/10.
- [16] S.H. Sicherer, A.W. Burks, H.A. Sampson, Clinical features of acute allergic reactions to peanut and tree nuts in children, *Pediatrics* 199:8(1):e6. Epub 1998/07/04. PubMed PMID: 9651458.
- [17] G. Du Toit, H.A. Sampson, M. Plaut, A.W. Burks, C.A. Akdis, G. Lack, Food allergy: Update on prevention and tolerance, *J. Allergy Clin. Immunol.* 141 (1) (2018) 30–40, <https://doi.org/10.1016/j.jaci.2017.11.010>. PubMed PMID: 29191680. Epub 2017/12/02.
- [18] G. Du Toit, Y. Katz, P. Sasieni, D. Mesher, S.J. Maleki, H.R. Fisher, A.T. Fox, V. Turcanu, T. Amir, G. Zadik-Mnuhin, A. Cohen, I. Livne, G. Lack, Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy, *J. Allergy Clin. Immunol.* 122 (5) (2008) 984–991, <https://doi.org/10.1016/j.jaci.2008.08.039>. PubMed PMID: 19000582.
- [19] G. Du Toit, G. Roberts, P.H. Sayre, H.T. Bahnson, S. Radulovic, A.F. Santos, H. A. Brough, D. Hippard, M. Basting, M. Feeney, V. Turcanu, M.L. Sever, M. Gomez Lorenzo, M. Plaut, G. Lack, L.S. Team, Randomized trial of peanut consumption in infants at risk for peanut allergy, *N. Engl. J. Med.* 372 (9) (2015) 803–813, <https://doi.org/10.1056/NEJMoa1414850>. PubMed PMID: 25705822; PMCID: 4416404.
- [20] G. Du Toit, P.H. Sayre, G. Roberts, M.L. Sever, K. Lawson, H.T. Bahnson, H. A. Brough, A.F. Santos, K.M. Harris, S. Radulovic, M. Basting, V. Turcanu, M. Plaut, G. Lack, Immune Tolerance Network L-OST. Effect of Avoidance on Peanut Allergy after Early Peanut Consumption, *N. Engl. J. Med.* 374 (15) (2016) 1435–1443, <https://doi.org/10.1056/NEJMoa1514209>. PubMed PMID: 26942922.
- [21] G. du Toit, P.H. Sayre, G. Roberts, K. Lawson, M.L. Sever, H.T. Bahnson, H. R. Fisher, M. Feeney, S. Radulovic, M. Basting, M. Plaut, G. Lack, Immune Tolerance Network Learning Early About Peanut Allergy study t. Allergen specificity of early peanut consumption and effect on development of allergic disease in the Learning Early About Peanut Allergy study cohort, *J. Allergy Clin. Immunol.* 141 (4) (2018) 1343–1353, <https://doi.org/10.1016/j.jaci.2017.09.034>. PubMed PMID: 29097103; PMCID: PMC5889963. Epub 2017/11/04.
- [22] M.R. Perkin, K. Logan, A. Tseng, B. Raji, S. Ayis, J. Peacock, H. Brough, T. Marrs, S. Radulovic, J. Craven, C. Flohr, G. Lack, Team EATS, Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants, *N. Engl. J. Med.* 374 (18) (2016) 1733–1743, <https://doi.org/10.1056/NEJMoa1514210>. PubMed PMID: 26943128. Epub 2016/03/05.
- [23] M. Suarez-Farinas, M. Suprun, H.T. Bahnson, R. Raghunathan, R. Getts, G. duToit, G. Lack, H.A. Sampson, Evolution of epitope-specific IgE and IgG4 antibodies in children enrolled in the LEAP trial, *J. Allergy Clin. Immunol.* 148 (3) (2021) 835–842, <https://doi.org/10.1016/j.jaci.2021.01.030>. PubMed PMID: 33592205; PMCID: PMC8480440. Epub 2021/02/17.
- [24] A. Winters, H.T. Bahnson, I. Ruczinski, M.P. Boorgula, C. Malley, A.R. Keramati, S. Chavan, D. Larson, K. Cerosaletti, P.H. Sayre, M. Plaut, G. Du Toit, G. Lack, K. C. Barnes, G.T. Nepom, R.A. Mathias, Immune Tolerance Network LST, The MALT1 locus and peanut avoidance in the risk for peanut allergy, *J. Allergy Clin. Immunol.* 143 (6) (2019) 2326–2329, <https://doi.org/10.1016/j.jaci.2019.02.016>. PubMed PMID: 30825465; PMCID: PMC6556406. Epub 2019/03/03.
- [25] K. Kanchan, S. Grinek, H.T. Bahnson, I. Ruczinski, G. Shankar, D. Larson, G. Du Toit, K.C. Barnes, H.A. Sampson, M. Suarez-Farinas, G. Lack, G.T. Nepom, K. Cerosaletti, R.A. Mathias, HLA alleles and sustained peanut consumption promote IgG4 responses in subjects protected from peanut allergy, *J. Clin. Investigation* 132 (1) (2022), <https://doi.org/10.1172/JCI152070>. PubMed PMID: 34981778; PMCID: PMC8718139. Epub 2022/01/05.
- [26] J.W. Krempski, J.K. Lama, K. Iijima, T. Kobayashi, M. Matsunaga, H. Kita, A mouse model of the LEAP study reveals a role for CTLA-4 in preventing peanut allergy induced by environmental peanut exposure, *J. Allergy Clin. Immunol.* (2022). Epub 2022/03/16., <https://doi.org/10.1016/j.jaci.2022.02.024>. PubMed PMID: 35288169.
- [27] T.R. Torgerson, A. Linane, N. Moes, S. Anover, V. Mateo, F. Rieux-Laucat, O. Hermine, S. Vijay, E. Gambineri, N. Cerf-Bensussan, A. Fischer, H.D. Ochs, O. Goulet, F.M. Ruemmele, 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. Epub 2007/05/09. doi: S0016-5085(07)00403-9 [pii] 10.1053/j.gastro.2007.02.044. PubMed PMID: 17484868.
- [28] R.A. Wood, S.H. Sicherer, B.P. Vickery, S.M. Jones, A.H. Liu, D.M. Fleischer, A. K. Henning, L. Mayer, A.W. Burks, A. Grishin, D. Stablein, H.A. Sampson, The natural history of milk allergy in an observational cohort, *J. Allergy Clin. Immunol.* 131 (3) (2013) 805–812. Epub 2013/01/01. doi: S0091-6749(12)01859-3 [pii] 10.1016/j.jaci.2012.10.060. PubMed PMID: 23273958; PMCID: 3691063.
- [29] S.H. Sicherer, R.A. Wood, B.P. Vickery, S.M. Jones, A.H. Liu, D.M. Fleischer, P. Dawson, L. Mayer, A.W. Burks, A. Grishin, D. Stablein, H.A. Sampson, The natural history of egg allergy in an observational cohort, *J. Allergy Clin. Immunol.* 133 (2) (2014) 492–9 e8, <https://doi.org/10.1016/j.jaci.2013.12.1041>. PubMed PMID: 24636473; PMCID: 3959659. Epub 2014/03/19.
- [30] R.L. Peters, I. Guarnieri, M.L.K. Tang, A.J. Lowe, S.C. Dharmage, K.P. Perrett, L. C. Gurrin, J.J. Koplin, The natural history of peanut and egg allergy in children up to age 6 years in the HealthNuts population-based longitudinal study, *J. Allergy Clin. Immunol.* (2022). Epub 2022/05/22. doi: 10.1016/j.jaci.2022.04.008. PubMed PMID: 35597613.
- [31] B. Ruiter, W.G. Shreffler, Innate immunostimulatory properties of allergens and their relevance to food allergy, *Semin. Immunopathol.* 34 (5) (2012) 617–632, <https://doi.org/10.1007/s00281-012-0334-8>. PubMed PMID: 22886110; PMCID: 3459673. Epub 2012/08/14.
- [32] M.R. Karlsson, J. Rugtveit, P. Brandtzaeg, Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow’s milk allergy, *J. Exp. Med.* 199 (12) (2004) 1679–1688. Epub 2004/06/16. doi: 10.1084/jem.20032121jem.20032121 [pii]. PubMed PMID: 15197226; PMCID: 2212808.
- [33] A.B. Fishbein, N. Qamar, K.A. Erickson, M.J. Kwasny, M. Cai, C. Szychlinski, A. M. Singh, R.L. Fuleihan, Cytokine responses to egg protein in previously allergic children who developed tolerance naturally. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*. 113 (6) (2014) 667–70 e4, <https://doi.org/10.1016/j.ana.2014.09.002>. PubMed PMID: 25282369.
- [34] N. Qamar, A.B. Fishbein, K.A. Erickson, M. Cai, C. Szychlinski, P.J. Bryce, R. P. Schleimer, R.L. Fuleihan, A.M. Singh, 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 : J. Br. Soc. Allergy Clin. Immunol.* 45 (11) (2015) 1663–1672, <https://doi.org/10.1111/cea.12570>. PubMed PMID: 25989379; PMCID: PMC4607624.
- [35] M.R. Neeland, J.J. Koplin, T.D. Dang, S.C. Dharmage, M.L. Tang, S.L. Prescott, R. Saffery, D.J. Martino, K.J. Allen, Early life innate immune signatures of persistent food allergy, *J. Allergy Clin. Immunology* 142 (3) (2018) 857–64 e3. Epub 2017/11/21. doi: 10.1016/j.jaci.2017.10.024. PubMed PMID: 29154959.
- [36] Dang TD, Peters RL, Koplin JJ, Dharmage SC, Gurrin LC, Ponsonby AL, Martino DJ, Neeland M, Tang MLK, Allen KJ, HealthNuts s. Egg allergen specific IgE diversity predicts resolution of egg allergy in the population cohort HealthNuts. *Allergy*. 2019;74(2):318–26. Epub 2018/07/24. doi: 10.1111/all.13572. PubMed PMID: 30035811.
- [37] D. Martino, M. Neeland, T. Dang, J. Cobb, J. Ellis, A. Barnett, M. Tang, P. Vuillermin, K. Allen, R. Saffery, Epigenetic dysregulation of naive CD4+ T-cell

- activation genes in childhood food allergy, *Nat. Commun.* 9 (1) (2018) 3308. Epub 20180817. doi: 10.1038/s41467-018-05608-4. PubMed PMID: 30120223; PMCID: PMC6098117.
- [38] D.J. Martino, A. Bosco, K.L. McKenna, E. Hollams, D. Mok, P.G. Holt, S.L. Prescott, T-cell activation genes differentially expressed at birth in CD4⁺ T-cells from children who develop IgE food allergy. *Allergy: European, J. Allergy Clin. Immunol.* 67 (2012) 191–200. <https://doi.org/10.1111/j.1398-9995.2011.02737.x>. PubMed PMID: 22077487.
- [39] B.P. Vickery, J.P. Berglund, C.M. Burk, J.P. Fine, E.H. Kim, J.I. Kim, C.A. Keet, M. Kulis, K.G. Orgel, R. Guo, P.H. Steele, Y.V. Virkud, P. Ye, B.L. Wright, R. A. Wood, A.W. Burks, Early oral immunotherapy in peanut-allergic preschool children is safe and highly effective, *J. Allergy Clin. Immunology*. 139 (1) (2017) 173–81 e8, doi: 10.1016/j.jaci.2016.05.027. PubMed PMID: 27522159; PMCID: PMC5222765.
- [40] B. Vickery, J. Lin, M. Kulis, P. Steele, S.M. Jones, A.M. Scurlock, L. Bardina, H. A. Sampson, A.W. Burks, The antibody response to peanut oral immunotherapy (OIT) is dynamic and individualized, *Clin. Exp. Allergy* 41 (12) (2011) 1851–1852. PubMed PMID: ISI:000297283800107.
- [41] B.P. Vickery, J. Lin, M. Kulis, Z. Fu, P.H. Steele, S.M. Jones, A.M. Scurlock, G. Gimenez, L. Bardina, H.A. Sampson, A.W. Burks, Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens, *J. Allergy Clin. Immunology* 131 (1) (2013), pp. 128–34 e1–3 Epub 2012/12/04. doi: S0091-6749 (12)01772-1 [pii] 10.1016/j.jaci.2012.10.048. PubMed PMID: 23199605; PMCID: 3529994.
- [42] M. Kulis, K. Saba, E.H. Kim, J.A. Bird, N. Kamilaris, B.P. Vickery, H. Staats, A. W. Burks, Increased peanut-specific IgA levels in saliva correlate with food challenge outcomes after peanut sublingual immunotherapy, *J. Allergy Clin. Immunology* 129 (4) (2012) 1159–1162. Epub 2012/01/13. doi: 10.1016/j.jaci.2011.11.045. PubMed PMID: 22236732; PMCID: PMC3763925.
- [43] B.L. Wright, M. Kulis, K.A. Orgel, A.W. Burks, P. Dawson, A.K. Henning, S. M. Jones, R.A. Wood, S.H. Sicherer, R.W. Lindblad, D. Stablein, D.Y. Leung, B. P. Vickery, H.A. Sampson, Consortium of Food Allergy R, Component-resolved analysis of IgA, IgE, and IgG4 during egg OIT identifies markers associated with sustained unresponsiveness, *Allergy*. 71 (11) (2016) 1552–1560. Epub 2016/03/27. doi: 10.1111/all.12895. PubMed PMID: 27015954; PMCID: PMC5035709.
- [44] A. Thyagarajan, S.M. Jones, A. Calatroni, L. Pons, M. Kulis, C.S. Woo, M. Kamalakannan, B.P. Vickery, A.M. Scurlock, A. Wesley Burks W.G. Shreffler, Evidence of pathway-specific basophil anergy induced by peanut oral immunotherapy in peanut-allergic children, *Clin. Exp. Allergy : J. Br. Soc. Allergy Clin. Immunology*. 42 (8) (2012) 1197–1205. Epub 2012/07/19. doi: 10.1111/j.1365-2222.2012.04028.x. PubMed PMID: 22805467; PMCID: 3779434.
- [45] Tsai M, Mukai K, Chinthrajah RS, Nadeau KC, Galli SJ. Sustained successful peanut oral immunotherapy associated with low basophil activation and peanut-specific IgE. *The Journal of allergy and clinical immunology*. 2020;145(3):885-96 e6. Epub 2019/12/06. doi: 10.1016/j.jaci.2019.10.038. PubMed PMID: 31805311; PMCID: PMC6957313.
- [46] Patil SU, Steinbrecher J, Calatroni A, Smith N, Ma A, Ruiter B, Virkud Y, Schneider M, Shreffler WG. Early decrease in basophil sensitivity to Ara h 2 precedes sustained unresponsiveness after peanut oral immunotherapy. *The Journal of allergy and clinical immunology*. 2019;144(5):1310-9 e4. Epub 2019/08/05. doi: 10.1016/j.jaci.2019.07.028. PubMed PMID: 31377342; PMCID: PMC6905043.
- [47] J.H. Savage, J.P. Courneya, P.M. Sterba, D.W. Macglashan, S.S. Saini, R.A. Wood, Kinetics of mast cell, basophil, and oral food challenge responses in omalizumab-treated adults with peanut allergy, *J. Allergy Clin. Immunology* 130 (5) (2012) 1123–9 e2. Epub 2012/07/18. doi: S0091-6749(12)00905-0 [pii] 10.1016/j.jaci.2012.05.039. PubMed PMID: 22800401.
- [48] J. Sun, K. Arias, D. Alvarez, R. Fattouh, T. Walker, S. Goncharova, B. Kim, S. Wasserman, J. Reed, A.J. Coyle, M. Jordana, Impact of CD40 ligand, B cells, and mast cells in peanut-induced anaphylactic responses, *J. Immunol.* 179 (10) (2007) 6696–6703. PubMed PMID: 17982059.
- [49] L.L. Reber, T. Marichal, K. Mukai, Y. Kita, S.M. Tokuoka, A. Roers, K. Hartmann, H. Karasuyama, K.C. Nadeau, M. Tsai, S.J. Galli, Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice, *J. Allergy Clin. Immunol.* 132 (4) (2013), 881–8 e1–11. Epub 2013/08/07. doi: 10.1016/j.jaci.2013.06.008. PubMed PMID: 23915716; PMCID: 3794715.
- [50] S.M. Jones, L. Pons, J.L. Roberts, A.M. Scurlock, T.T. Perry, M. Kulis, W. G. Shreffler, P. Steele, K.A. Henry, M. Adair, J.M. Francis, S. Durham, B.P. Vickery, X. Zhong, A.W. Burks, Clinical efficacy and immune regulation with peanut oral immunotherapy, *J. Allergy Clin. Immunol.* 124 (2) (2009) 292–300, e1–97. Epub 2009/07/07. doi: S0091-6749(09)00813-6 [pii] 10.1016/j.jaci.2009.05.022. PubMed PMID: 19577283; PMCID: 2725434.
- [51] M. Gorelik, S.D. Narisety, A.L. Guerrero, K.L. Chichester, C.A. Keet, A. P. Bieneman, R.G. Hamilton, R.A. Wood, J.T. Schroeder, P.A. Frischmeyer-Guerrero, Suppression of the immunologic response to peanut during immunotherapy is often transient, *J. Allergy Clin. Immunology*. 135 (5) (2015) 1283–1292. Epub 2014/12/30. doi: 10.1016/j.jaci.2014.11.010. PubMed PMID: 25542883; PMCID: PMC4426213.
- [52] Bajzik V, DeBerg HA, Garabatos N, Rust BJ, Obrien KK, Nguyen QA, O'Rourke C, Smith A, Walker AH, Quinn C, Gersuk VH, Farrington M, Jeong D, Vickery BP, Adelman DC, Wambre E. Oral desensitization therapy for peanut allergy induces dynamic changes in peanut-specific immune responses. *Allergy*. 2022. Epub 2022/03/11. doi: 10.1111/all.15276. PubMed PMID: 35266148.
- [53] E. Wambre, V. Bajzik, J.H. DeLong, K. O'Brien, Q.A. Nguyen, C. Speake, V. H. Gersuk, H.A. DeBerg, E. Whalen, C. Ni, M. Farrington, D. Jeong, D. Robinson, P. S. Linsley, B.P. Vickery, W.W. Kwok, A phenotypically and functionally distinct human TH2 cell subpopulation is associated with allergic disorders, *Sci. Transl. Med.* 9 (401) (2017), <https://doi.org/10.1126/scitranslmed.aam9171>. PubMed PMID: 28768806.
- [54] M.C. Berin, C. Agashe, A.W. Burks, D. Chiang, W.F. Davidson, P. Dawson, A. Grishin, A.K. Henning, S.M. Jones, E.H. Kim, D.Y.M. Leung, M. Masilamani, A. M. Scurlock, S.H. Sicherer, R.A. Wood, H.A. Sampson, Allergen-specific T cells and clinical features of food allergy: Lessons from CoFAR immunotherapy cohorts, *J. Allergy Clin. Immunology*. 149 (4) (2022) 1373–1382, e12. Epub 2021/10/16. doi: 10.1016/j.jaci.2021.09.029. PubMed PMID: 34653515; PMCID: PMC8995337.
- [55] B. Monian, A.A. Tu, B. Ruiter, D.M. Morgan, P.M. Petrossian, N.P. Smith, T. M. Gierahn, J.H. Ginder, W.G. Shreffler, J.C. Love, Peanut oral immunotherapy differentially suppresses clonally distinct subsets of T helper cells, *J. Clin. Investigation* 132 (2) (2022). Epub 2021/11/24. doi: 10.1172/JCI150634. PubMed PMID: 34813505; PMCID: PMC8759778.
- [56] M. Kulis, X. Yue, R. Guo, H. Zhang, K. Orgel, P. Ye, Q. Li, Y. Liu, E. Kim, A. W. Burks, B.P. Vickery, High- and low-dose oral immunotherapy similarly suppress pro-allergic cytokines and basophil activation in young children, *Clin. Exp. Allergy : J. Br. Soc. Allergy Clin. Immunology*. 49 (2) (2019) 180–189. Epub 20180918. doi: 10.1111/cea.132PubMed PMID: 30126028; PMCID: PMC6353702.
- [57] P. Varshney, S.M. Jones, A.M. Scurlock, T.T. Perry, A. Kemper, P. Steele, A. Hiegel, J. Kamilaris, S. Carlisle, X. Yue, M. Kulis, L. Pons, B. Vickery, A.W. Burks, A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response, *J. Allergy Clin. Immunology*. 127 (3) (2011) 654–660. Epub 2011/03/08. doi: S0091-6749(11) 00050-9 [pii] 10.1016/j.jaci.2010.12.1111. PubMed PMID: 21377034; PMCID: 3060783.
- [58] Lozano-Ojalvo D, Tyler SR, Aranda CJ, Wang J, Sicherer S, Sampson HA, Wood RA, Burks AW, Jones SM, Leung DYM, Curotto de Lafaille M, Berin MC. Allergen recognition by specific effector Th2 cells enables IL-2-dependent activation of regulatory T cell responses in humans. *medRxiv*. 2022:2022.05.17.22275017. doi: 10.1101/2022.05.17.22275017.
- [59] Syed A, Garcia MA, Lyu SC, Bucayu R, Kohli A, Ishida S, Berglund JP, Tsai M, Maecker H, O'Riordan G, Galli SJ, Nadeau KC. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *The Journal of allergy and clinical immunology*. 2014;133(2):500-10 e11. Epub 2014/03/19. doi: 10.1016/j.jaci.2013.12.1037. PubMed PMID: 24636474.
- [60] J.F. Ryan, R. Hovde, J. Glanville, S.C. Lyu, X. Ji, S. Gupta, R.J. Tibshirani, D.C. Jay, S.D. Boyd, R.S. Chinthrajah, M.M. Davis, S.J. Galli, H.T. Maecker, K.C. Nadeau, Successful immunotherapy induces previously unidentified allergen-specific CD4⁺ T-cell subsets, *Proc Natl Acad Sci U S A*. 113 (9) (2016) E1286–E1295, <https://doi.org/10.1073/pnas.1520180113>. PubMed PMID: 26811452; PMCID: PMC4780622.
- [61] Wang W, Lyu SC, Ji X, Gupta S, Manohar M, Dhondalay GKR, Chinthrajah S, Andorf S, Boyd SD, Tibshirani R, Galli SJ, Nadeau KC, Maecker HT. Transcriptional changes in peanut-specific CD4⁺ T cells over the course of oral immunotherapy. *Clin Immunol.* 2020;219:108568. Epub 2020/08/14. doi: 10.1016/j.clim.2020.108568. PubMed PMID: 32783912; PMCID: PMC7484319.
- [62] Zhang W, Dhondalay GK, Liu TA, Kaushik A, Hoh R, Kwok S, Kambham N, Fernandez-Becker NQ, Andorf S, Desai M, Galli SJ, Boyd SD, Nadeau KC, Manohar M, DeKruyff RH, Chinthrajah RS. Gastrointestinal gammadelta T cells reveal differentially expressed transcripts and enriched pathways during peanut oral immunotherapy. *Allergy*. 2022;77(5):1606-10. Epub 2022/02/11. doi: 10.1111/all.15250. PubMed PMID: 35143054.
- [63] MacGinnitie AJ, Rachid R, Gragg H, Little SV, Lakin P, Cianferoni A, Heimall J, Makhija M, Robison R, Chinthrajah RS, Lee J, Lebovidge J, Dominguez T, Rooney C, Lewis MO, Koss J, Burke-Roberts E, Chin K, Logvinenko T, Pongracic JA, Umetsu DT, Spergel J, Nadeau KC, Schneider LC. Omalizumab facilitates rapid oral desensitization for peanut allergy. *The Journal of allergy and clinical immunology*. 2017;139(3):873-81 e8. doi: 10.1016/j.jaci.2016.08.010. PubMed PMID: 27609658; PMCID: PMC5369605.
- [64] F. van Wijk, S. Hoeks, S. Nierkens, S.J. Koppelman, P. van Kooten, L. Boon, L. M. Knippels, R. Pieters, CTLA-4 signaling regulates the intensity of hypersensitivity responses to food antigens, but is not decisive in the induction of sensitization, *J. Immunol.* 174 (1) (2005) 174–179. PubMed PMID: 15611239.
- [65] H. Maazi, S. Shirinbak, L.E. den Boef, F. Fallarino, C. Volpi, M.C. Nawijn, A.J. van Oosterhout, Cytotoxic T lymphocyte antigen 4-immunoglobulin G is a potent adjuvant for experimental allergen immunotherapy, *Clin. Exp. Immunol.* 172 (1) (2013) 113–120, <https://doi.org/10.1111/cei.12041>. PubMed PMID: 23480191; PMCID: PMC3719937.