

Immunology of Food Allergy

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Many consider food allergy as the “second wave” of the allergy epidemic following the “first wave” of respiratory allergy, i.e., asthma and allergic rhinitis, plaguing westernized countries, with up to 8% of young children and 2%–3% of adults in the United States now affected by hypersensitivity reactions to various foods. In the past decade, there have been great strides in our understanding of the underlying immunopathogenesis of these disorders, which have led to improved diagnostic techniques, management strategies, and therapeutic approaches. Here we will review the most recent understanding of basic mechanisms underlying IgE-mediated food allergies and novel therapeutic approaches under investigation for both the prevention and treatment of IgE-mediated food allergies.

Introduction

Food allergy is defined as an immune-mediated adverse reaction to food. This definition includes IgE-mediated immediate hypersensitivity reactions, delayed non-IgE-mediated reactions, and disorders with contributions from both IgE-mediated and non-IgE-mediated immune pathways. Up to 8% of children in the United States are now believed to be affected by food allergies (Gupta et al., 2011), and several studies suggest that there was a major increase since the late 1990s. Skin and food allergies are emerging as a “second wave” of the allergy epidemic, replacing the “first wave” dominated by asthma and allergic rhinitis, which began in the 1950s (Prescott and Allen, 2011). In this review, we will focus exclusively on the IgE-mediated food allergies, for which there is a greater depth of understanding of immune mechanisms. Reactions are elicited rapidly (minutes up to 2 hr) after ingestion of the offending food, and symptoms can affect the skin (hives), respiratory tract (wheezing, coughing), and gastrointestinal tract (nausea, vomiting, diarrhea). The term anaphylaxis refers to a severe allergic reaction that affects two or more organ systems. In this review, we will discuss recent research progress in understanding the immune mechanisms of IgE-mediated food allergies.

Mechanisms of Food-Induced Anaphylaxis

There is evidence from human studies and mouse models of food-induced anaphylaxis that basophils and mast cells contribute to allergic reactions to foods (Reber et al., 2013; Savage et al., 2012; Sun et al., 2007). Although histamine is elevated and contributes to allergic symptoms, platelet activating factor (PAF) is uniquely associated with anaphylaxis severity in mice and humans (Arias et al., 2009; Vadas et al., 2008, 2013). Interestingly, PAF is produced not only by mast cells and basophils, but also by other cell types such as macrophages or neutrophils that contribute to alternative pathways of anaphylaxis. In mice, high allergen exposure elicits activation of basophils, neutrophils, and macrophages through IgG-antigen immune complexes acting on FcγRIII and FcγRIV (Finkelman et al., 2016; Jönsson et al., 2011; Strait et al., 2002; Tsujimura et al., 2008). Although IgG-mediated activation of neutrophils has been documented using human blood (Khodoun et al., 2011), the possible role of this pathway in food allergy is not clear.

We have used mass cytometry (CyTOF) to identify cell activation in all major cell types found in peripheral blood of peanut allergic subjects following a short (15–30 min) *in vitro* exposure to peanut allergen (Tordesillas et al., 2016). In addition to strong activation of basophils after peanut exposure as assessed by traditional activation markers (CD63), we observed evidence of activation in monocytes (phospho-S6), dendritic cells (DCs, also phospho-S6), and neutrophils (downregulation of CD66a). This observation was unique to peanut-allergic subjects after peanut stimulation, but could also be elicited in healthy controls by cross-linking of IgE, indicating activation through IgE-dependent mechanisms (Tordesillas et al., 2016). These data show that not only basophils, but also myeloid cells, may contribute to early stages of allergic reactions. Innate inflammatory pathways have also been identified as activated in whole blood of patients undergoing anaphylaxis, as detected using analysis of transcriptional changes measured by microarray. TNFα, LPS, and TLR signaling pathways were identified as enriched in the differentially expressed genes associated with anaphylaxis (Stone et al., 2014). We observed a similar innate inflammatory response in peripheral blood mononuclear cells (PBMCs) of egg-allergic subjects stimulated *in vitro* with egg allergen, with a dominant toll-like receptor 4 (TLR4) and monocyte activation signature (Kosoy et al., 2016). Neutrophil activation markers (myeloperoxidase) have also been identified in peripheral blood of those presenting to the emergency room with anaphylaxis from various triggers, including foods (Francis et al., 2017). These data point to a novel role for myeloid cells, including neutrophils and monocytes, in the acute allergic response to foods. However, the receptors involved in the activation of innate inflammatory pathways during allergen exposure need to be investigated.

In addition to activation of myeloid cells following stimulation with peanut, we have identified an interaction of basophils with platelets after stimulation with peanut in peanut-allergic but not healthy subjects (Tordesillas et al., 2016). There is older literature from mouse models suggesting that platelets can contribute to anaphylaxis severity (Pinckard et al., 1977). Thus, the cross-talk between basophils and platelets may be another novel mechanism by which PAF may contribute to anaphylaxis severity.

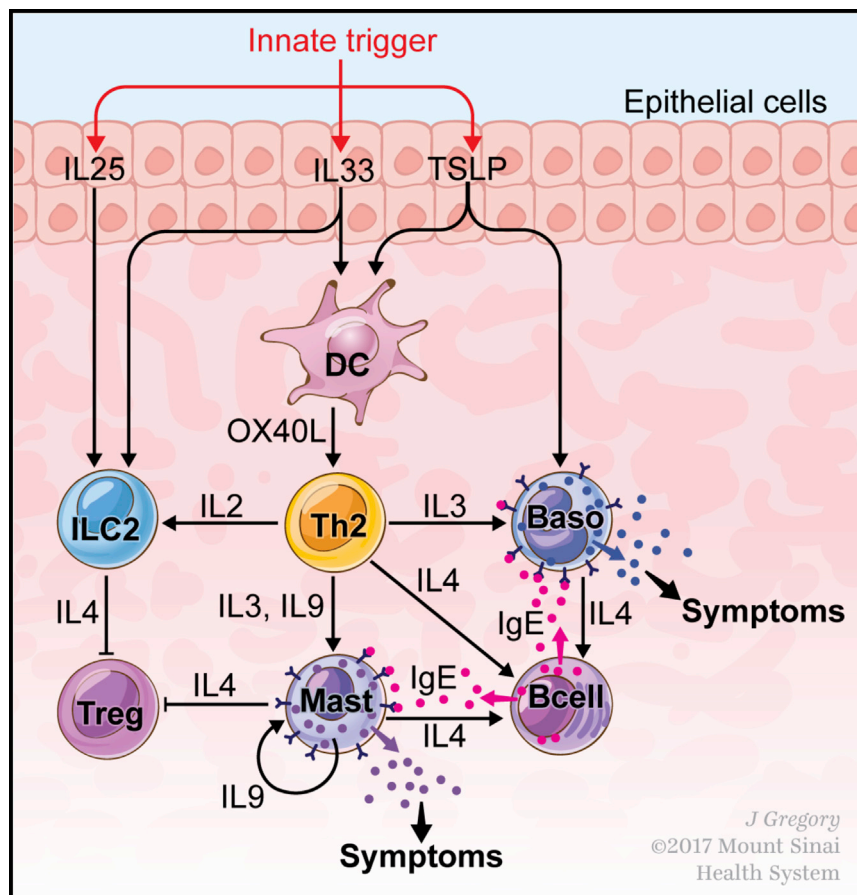


Figure 1. Innate Immune Mechanisms Contributing to Sensitization

Factors triggering innate responses, such as damage or adjuvant activity, promote production of cytokines by epithelial cells, acting on innate immune cells such as ILC2s, DCs, and basophils. DCs upregulate OX40L, priming T cells toward a Th2 cell responses. ILC2s and basophils are involved in an amplification loop of Th2 cell responses by producing Th2 cytokines. Th2 cells promote IgE class switching on B cells through production of IL-4. IgE on the membrane of mast cells and basophil is responsible for clinical symptoms after antigen cross-linking. IL-9 is crucial for the expansion of mast cells that contribute to food allergy symptoms. Mast cells and ILC2s contribute to the suppression of tolerance responses by production of IL-4.

eczematous skin through the use of tape-stripping, topical vitamin D analogs, or intradermal injections of the cytokine thymic stromal lymphopoietin (TSLP) (Han et al., 2014; Leyva-Castillo et al., 2013; Oyoshi et al., 2010; Tordesillas et al., 2014, 2017). Limitations common to most of the model systems include strain specificity, the need for extreme doses to elicit symptoms on oral challenge, or the need for systemic allergen challenge. The recent development of humanized mouse systems, utilizing either an engrafted human immune system derived from human hematopoietic stem cells (Bryce et al., 2016; Burton et al., 2017) or transgenic expres-

sion of human IgE receptors (Platzer et al., 2015), represents a step toward models that more accurately reflect the human response to food allergens.

Tissue Mechanisms of Sensitization to Foods

The default response to innocuous antigens is one of immune tolerance, which is mediated by presentation of antigen by CD103⁺ DCs in the gastrointestinal tract. CD11b⁺ dermal DCs and Langerhans cells (LCs) have also been shown to mediate immune tolerance in the skin. In the context of food allergy, the development of regulatory T (Treg) cells is believed to be compromised and replaced by the generation of T helper 2 (Th2) cells that drive IgE class switching and the expansion of allergic effector cells. There has been much focus on the factors responsible for this deviated immune response, and a summary of these findings is shown in Figure 1.

In murine models in which exogenous adjuvants are used to break oral tolerance, IL-33 expression by intestinal epithelial cells drives OX40L expression on CD103⁺ intestinal DCs, resulting in Th2 cell skewing (Blázquez and Berin, 2008; Chu et al., 2013). IL-33 has also been shown to be central to the pathogenesis of food allergy elicited through the skin. Topical application of peanut, which has been previously identified to have innate stimulatory activity on human DCs (Shreffler et al., 2006), upregulates IL-33 expression from keratinocytes and drives Th2 cell skewing by skin-draining DCs by a mechanism dependent on ST2, the IL-33 receptor (Tordesillas et al., 2014).

Mechanism of Sensitization to Foods Experimental Model Systems

Much of our understanding of mechanisms of sensitization to foods comes from experimental model systems in mice, which are broadly divided into two categories: those that arise from oral sensitization and those that arise from topical or epicutaneous sensitization. Mice normally develop oral tolerance to antigens delivered by the oral route, and therefore adjuvants such as cholera toxin or staphylococcal enterotoxin B (SEB) are used to break oral tolerance (Ganeshan et al., 2009). SEB is a weaker adjuvant, particularly by the oral route, but is more likely to be a factor in human sensitization than cholera toxin, due to the presence of toxin-producing *Staphylococcus aureus* strains colonizing the skin of patients with eczematous lesions (Monti et al., 1996). As will be discussed later, early onset and severity of eczema has been directly associated with increased risk of food allergies (Martin et al., 2015).

Genetic susceptibility to develop allergic sensitization has been modeled with transgenic mice carrying a gain-of-function mutation in the IL-4R α chain (il4raF09) (Burton et al., 2014b). These mice are prone to develop food allergy by oral sensitization in the absence of adjuvant and show enhanced responsiveness to the weak adjuvant SEB. In response to growing clinical evidence for the role of the skin in sensitization to peanut, a number of models utilizing epicutaneous sensitization have been developed. These include adjuvant-free models and models that mimic

IL-33 is also increased by damage in mouse or human skin (Galand et al., 2016). Another pathway by which IL-33 promotes food allergy is through expansion and activation of group 2 innate lymphoid cells (ILC2s). In the *il4raF09* mouse model, ILC2s respond to IL-33 by producing large amounts of IL-4 that suppress the generation of Treg cells in the small intestine (Noval Rivas et al., 2016). In addition to effects on Th2 cell priming and sensitization, IL-33 contributes to acute reactions to food by acting directly on mast cells and enhancing IgE-mediated activation (Galand et al., 2016).

Atopic dermatitis, which is a strong risk factor for food allergy, is associated with elevated expression of the Th2-inducing cytokine TSLP (Soumelis et al., 2002). Elevated TSLP expression in mouse skin is driven by tape stripping or topical vitamin D analogs, and topical application of antigen to the skin in these models results in sensitization through a TSLP-dependent mechanism (Han et al., 2014; Noti et al., 2013; Oyoshi et al., 2010). TSLP can promote sensitization through dendritic cells and basophils (Han et al., 2014; Noti et al., 2013). OX40L-expressing DCs recruit basophils into the draining lymph nodes through the induction of IL-3-secreting CD4⁺ T cells. Basophils then contribute by producing IL-4 and promoting IL-4 production from CD4⁺ T cells (Leyva-Castillo et al., 2013). TSLP can also act directly on basophils (Siracusa et al., 2011).

In addition to TSLP and IL-33, IL-25 is the third of a trio of Th2-inducing cytokines that can contribute to sensitization to foods. IL-25 is upregulated in the intestine of mice with food allergy. Transgenic overexpression of IL-25 in the intestine enhanced experimental food allergy, while mice lacking the IL-25 receptor were resistant to food allergy (Han et al., 2014; Lee et al., 2016). IL-25 induced a CD4⁺ T cell-dependent induction of Th2 cell-type cytokine release from ILC2s that amplified intestinal inflammation. In this model, ILC2s were functioning downstream of CD4⁺ T cells to amplify rather than initiate a CD4⁺ T cell-mediated Th2 response (Lee et al., 2016).

IL-9, Mast Cells, and the Link to Regulatory T Cells

The Th2 cell-associated cytokine IL-9 has recently emerged as a key cytokine associated with the allergic response to foods in humans and mouse models. IL-9 was the most highly differentially expressed gene in peanut-activated memory CD4⁺ T cells from peanut-allergic subjects compared to non-allergic subjects (Brough et al., 2014a). IL-9 was also identified as differentially expressed and secreted from PBMCs from egg-allergic subjects but not atopic controls in response to egg (Kosoy et al., 2016). IL-9 is a growth factor for mast cells and has been shown in mouse models to be essential in the pathogenesis of food allergy. IL-9 deficiency prevents experimental food allergy, while transgenic overexpression in the intestinal epithelium induces intestinal mastocytosis, increased epithelial permeability, and increased susceptibility to food allergy (Forbes et al., 2008; Osterfeld et al., 2010). A population of mucosal mast cells that produce high amounts of IL-9 and IL-13 was identified (Chen et al., 2015). This MMC9 population was induced by Th2 cells and promotes the expansion of mature granulated mast cells in the intestine as well as symptoms after oral challenge. The role of this population in human disease was supported by the finding of elevated IL-9, IL-13, tryptase, chymase, and carboxypeptidase gene expression in the duodenum of food-allergic patients in comparison with control subjects (Chen et al., 2015).

In addition to producing cytokines such as IL-9 and IL-13 that can contribute to intestinal allergic inflammation, there is an interesting bi-directional communication between mast cells and Treg cells. Activation of mast cells through IgE leads to a suppression of Treg cell generation and amplification of Th2 cell responses (Burton et al., 2014b). Transfer studies with IL-4-deficient mast cells demonstrated a key role for mast cell-derived IL-4 in the pathogenesis of food allergy. Others have reported similar key roles for cytokine-producing mast cells in driving Th2 cell inflammation in a model of peanut allergy (Wang et al., 2010b). Further investigation of the impact of mast cells in Treg cells demonstrated not only a deficiency in Treg cell generation but also a reprogramming such that Foxp3⁺ Treg cells were expressing IL-4, were poorly suppressive, and in fact contributed to food allergy pathogenesis (Noval Rivas et al., 2015). Furthermore, transfer of Treg cells from mice without genetic susceptibility to food allergy could suppress food allergy symptoms in mice. Even in established food allergy, Treg cells can inhibit symptoms by direct suppression of mast cell activation (Ganeshan and Bryce, 2012; Tordesillas et al., 2017).

A role for Treg cells in food allergy has also been supported by data from human subjects. Reprogrammed Treg cells co-expressing IL-4 and IL-13 were detected in PBMCs from individuals with milk allergy. PBMCs from milk-allergic subjects cultured with β -lactoglobulin for 5 days and re-stimulated with PMA and ionomycin contained a population of Foxp3⁺CD4⁺ T cells that co-expressed IL-4 and IL-13 (Noval Rivas et al., 2015). This was not observed in PBMCs from healthy controls or in those with peanut allergy but no milk allergy. In children who have naturally outgrown their food allergy, an increase in Foxp3⁺ Treg cells and IL-10-producing CD4⁺ T cells after antigen stimulation was described (Qamar et al., 2015), while the expression of IL-5 by Foxp3⁺ T cells was reduced compared to allergic subjects and healthy controls. This suggests that a population of non-Th2-like Treg cells may be required for developing tolerance to foods.

The Food-Specific Antibody Response

Production of allergen-specific IgE is central to the development of food allergies. The mechanism and site of induction of IgE responses in the context of food allergy is poorly understood. In germ-free mice, which have high basal levels of IgE, IgE class-switching has been identified in Peyer's patches, and replenishing the microbiota was necessary to restore normal low levels of IgE (Cahenzli et al., 2013). However, whether the Peyer's patch is also the site of IgE class-switching during sensitization to foods remains to be determined. Recently, it has been proposed that long-lived memory B cells are the main source that maintains IgE titers in food allergy and they required continued antigen exposure and cooperation of CD4⁺ T cells (Jiménez-Saiz et al., 2017). By contrast, when B cells were depleted using an anti-CD20 antibody in a model of peanut allergy, antigen-specific IgE levels were not affected, suggesting that long-lived IgE⁺ plasma cells were responsible for persistent serum IgE levels observed in peanut-allergic mice (Moutsoglou and Dreskin, 2016). So, it is possible that both memory B cells and long-lived IgE⁺ plasma cells contribute to persistent antigen-specific IgE production.

Despite the fact that IgE plays a central role in the development of food allergies, the measurement of food-specific IgE in

sera does not always discriminate between those subjects that are only sensitized from those who are truly allergic. In addition, the presence of conserved proteins in different plant species can lead to cross-reactivity, which does not always lead to clinical reactivity to the cross-reactive food. Component resolved diagnosis, in which recombinant or native purified allergenic proteins are used for measuring specific IgE instead of whole food extracts, has been implemented in the last few years to improve the specificity of diagnosis and, in some cases, to obtain a better prediction of the clinical phenotype.

The best example of a better diagnostic using IgE concentrations to component proteins rather than to the whole extract is the case of peanut. IgE against the major allergen Ara h 2 is a better discriminator of allergy or tolerance to peanut than peanut-specific IgE and is associated with greater severity of symptoms (Beyer et al., 2015; Nicolaou et al., 2011). On the contrary, monosensitization to Ara h 8, which is homologous to the major birch allergen Bet v 1, is correlated with tolerance to peanut or mild oral-pharyngeal symptoms (Lieberman et al., 2013). The correlation between allergen-specific IgE and clinical phenotype varies among geographical regions. One example is the peanut lipid transfer protein Ara h 9, which is a marker for severity of peanut allergy in southern Europe (Sastre, 2010). For a list of the components of the major food allergens, see Table 1.

The correlation between IgE to component proteins and allergy to other foods is not as clear as in the case of peanut, hazelnut, or cashew. In egg allergy, IgE antibodies against ovomucoid have been found to be predictive of reactivity to extensively heated forms of egg. For milk allergy, high levels of casein-specific IgE have been correlated with persistent milk allergy (Boyano-Martínez et al., 2009) and reactivity to baked milk (Caubet et al., 2013). In the case of hazelnut, IgE antibodies to Cor a 9 and Cor a 14 were found to be the best to distinguish systemic allergy from oral symptoms to hazelnut (Beyer et al., 2015; Masthoff et al., 2013) and Ana o 3 was optimal for detecting systemic reactions to cashew and closely related pistachio (Savvastianos et al., 2015).

The epitope diversity of the food-specific IgE response can also be informative of the clinical reactivity. Binding to a greater number of sequential allergenic epitopes (IgE-binding peptides) correlated with severity of allergic reactions and persistence of milk allergy (Wang et al., 2010a). Recognition of sequential and conformational epitopes also distinguishes between different clinical phenotypes. In the case of milk and egg allergy, recognition of predominantly conformational epitopes is associated with a transient allergy, which is thought to be related to higher intestinal permeability in young children that allows intact proteins to cross the intestinal barrier (Järvinen et al., 2002, 2007). Most children who are allergic to milk or egg tolerate extensively heated (baked) forms of these foods, where conformational epitopes are destroyed. By contrast, recognition of sequential epitopes in milk and egg allergens is associated with persistence of the milk or egg allergy. Other food allergens such as lipid transfer proteins, which are important allergens of Rosaceae fruits, peanuts, tree nuts, and cereals among others, or conglutins, such as the major peanut allergens Ara h 2 and Ara h 6, have been described to be highly resistant to high temperature and gastrointestinal digestion (Apostolovic et al., 2016; Egger et al., 2010). While IgE antibody binding to an increasing number of sequential allergenic

epitopes has been associated with increasing severity of allergic reactions (Flinterman et al., 2008; Shreffler et al., 2005), the role of conformational epitopes in the induction of anaphylactic reactions to these allergens remains to be established.

The Skin as a Site of Food-Allergen Sensitization

There is a growing body of evidence in infants suggesting that the main site of sensitization to foods, particularly peanut, occurs in the skin. It has long been appreciated that eczema is a major risk factor for food allergies (Loo et al., 2016; Martin et al., 2015). In one of the first studies examining risk factors for the development of peanut allergy, the use of diaper creams in infants containing peanut oil was found to be associated with peanut allergy (Lack et al., 2003). Similarly, the use of facial soap containing wheat protein was also identified as a risk for wheat allergy (Fukutomi et al., 2014). Mutations in filaggrin, a protein essential to maintain skin barrier, have been associated with increased risk of atopic dermatitis as well as food allergy (Brown et al., 2011; Venkataraman et al., 2014). Epidemiologic evidence shows that environmental exposure to peanut proteins present in house dust is a risk factor for the development of peanut allergy, particularly in those with filaggrin mutations (Brough et al., 2014b; Fox et al., 2009). In support of the hypothesis that sensitization occurs through the skin, peanut-specific T cells from peanut-allergic patients are found in the CLA⁺ compartment (skin homing) but not the $\beta 7^+$ compartment (gut homing) (Chan et al., 2012; DeLong et al., 2011). Similar findings were reported earlier in young children with atopic dermatitis and milk allergy (Abernathy-Carver et al., 1995). Given that the initial site of priming imprints homing capacity to the gastrointestinal tract and skin, these data support the hypothesis that primary sensitization to peanut and milk may occur through the skin. Whether this is true for other food allergens still needs to be addressed.

The plausibility of skin sensitization to foods has been demonstrated in numerous mouse models. We have shown that peanut has intrinsic adjuvant activity that promotes sensitization upon topical exposure (Tordesillas et al., 2014). Tape stripping, which is a model of damage induced by scratching in eczema, facilitates sensitization through the skin (Jin et al., 2009; Oyoshi et al., 2010). Staphylococcal enterotoxin B (SEB), a toxin present at increased frequency in lesional skin in eczema, also promotes bystander sensitization to food antigens (Tordesillas et al., 2014). This supports the concept of sensitization through eczematous skin.

Oral Tolerance and the Prevention of Food Allergy

The “dual exposure hypothesis” of food allergy proposes that whereas exposure through eczematous skin promotes sensitization to foods, early oral exposure promotes tolerance (Lack, 2012). Studies in mice have clearly shown that oral exposure results in tolerance, mediated by induced Foxp3⁺ Treg cells that suppress the development of IgE and allergy. CD103⁺ DCs acquiring antigen in the unique immune milieu of the small intestine prime naive T cells to become regulatory through mechanisms involving TGF- β and retinoic acid (Hadis et al., 2011; Johansson et al., 2011; Shan et al., 2013). Local intestinal factors, such as GM-CSF produced by innate lymphoid cells (Mortha et al., 2014) and mucin produced by epithelial cells (Shan et al., 2013), program intestinal dendritic cells with a default regulatory phenotype. A schematic of the dual exposure hypothesis is shown in Figure 2. Epidemiologic evidence supported the

hypothesis that early dietary introduction is tolerizing, as it was observed that consumption of foods such as peanut, milk, fish, or wheat in early life has been associated with a reduced incidence of food allergy (Du Toit et al., 2008; Katz et al., 2010; Kull et al., 2006; Poole et al., 2006). To test whether this association was causal, several randomized controlled trials have been performed to analyze the effect of early introduction of foods in the development of food allergy (Table 2). In the LEAP trial, the investigators sought to determine whether early oral introduction of peanut into the diet could decrease the development of peanut allergy. Thus, children at increased risk of peanut allergy were randomized whether or not to introduce peanut into the diet between the ages of 4 and 11 months. Early introduction of peanut resulted in a dramatic reduction in prevalence of peanut allergy (Du Toit et al., 2015) and this protection remained even after cessation of peanut consumption for 12 months (Du Toit et al., 2016). In the case of egg, the results have not been consistent. Early introduction of egg into the diet of children was poorly tolerated by a significant proportion of children who were already sensitized and clinically reactive to egg at 4 months of age (Bellach et al., 2017; Palmer et al., 2013), although the proportion was reduced when considering only children without eczema (Palmer et al., 2017). Authors concluded that adding egg to the diet at 4–6 months of age was not effective in reducing the development of egg allergy (Bellach et al., 2017; Palmer et al., 2013, 2017). Positive results in a high-risk cohort were recently reported from the PETIT trial using early introduction of egg to 4- to 6-month-old children (Natsume et al., 2017). This study used a lower dose of egg at introduction (50 mg versus 900 or 2,500 mg of egg protein) and stepped up to 250 mg after 3 months. Other key differences in the PETIT trial included using heated rather than pasteurized raw egg powder, which lowers the allergenicity of the egg protein, and aggressively treating eczema. The fact that many of the children were already sensitized at the beginning of the study suggests that the treatment could be acting more as a therapeutic intervention than as a prevention. The EAT study attempted to broaden the impact of early introduction by increasing the number of foods to six and using a cohort that was not enriched for those at elevated risk of food allergy (Perkin et al., 2016). Although there was some efficacy for egg and peanut in the “per protocol” analysis, the low adherence to the trial protocol resulted in a non-significant impact of intervention in the primary outcome (Perkin et al., 2016). The LEAP findings have recently resulted in a change in recommendations on introduction of peanut into the diet (Togias et al., 2017). In 2000, the American Academy of Pediatrics had recommended that introduction of highly allergenic foods be delayed until 3 years in infants with increased risk of allergy. In 2008 those guidelines were revised to state that introduction of highly allergenic foods need not be delayed beyond 4–6 months of age, but they did not specifically recommend early introduction. At the beginning of this year, the guidelines were changed to recommend early introduction of peanut, with the recommendation that high-risk infants seek evaluation from an allergy specialist prior to introduction (Togias et al., 2017). It remains to be seen whether this reversal in feeding guidelines will reduce the incidence of peanut allergy.

Studies examining the immune mechanisms of early introduction of foods have been limited to date. The LEAP study

observed a highly significant induction of peanut-specific IgG4 in response to inclusion of peanut (Du Toit et al., 2015). The PETIT study observed a significant elevation of ovomucoid-specific IgG1, IgG4, and IgA and a reduction of ovomucoid-specific IgE in response to early dietary introduction of egg (Natsume et al., 2017). The induction of blocking antibodies is associated with and likely contributes to the reduction in clinical reactivity. Allergen-specific T cell responses have not yet been addressed in this context.

Microbiome and the Regulation of Food Allergy

The rapid increase in food allergy prevalence in the last two decades indicates that environmental factors modify risk. Risk factors related to environment include mode of delivery (elevated risk associated with cesarean delivery), exposure to pets or farm environment (protective), having older siblings (protective), and exposure to daycare in early life (protective). These factors are associated with changes in the intestinal microbiota, and therefore may exert their influence by microbial influence on the mucosal immune system. There is growing evidence for a dysbiosis in patients with food allergy (Hua et al., 2015; Ling et al., 2014), which may precede the onset of disease (Azad et al., 2015). Differences in microbial composition have also been linked to resolution of milk allergy (Bunyavanich et al., 2016). Limitations of studies examining the association of microbiome with food allergy to date include small sample size and/or the use of sensitization or self-report as a measure of food allergy. Although several studies have identified differences in microbial composition between food allergy and controls, there is a lack of information on the functional consequence of changes in microbial composition. Dysbiosis in the context of asthma has been mechanistically linked to immune regulation, clearly demonstrating microbial-induced changes in innate or adaptive immunity that underlie the asthmatic phenotype (Arrieta et al., 2015; Fujimura et al., 2016; Stein et al., 2016). Furthermore, protective bacterial strains and microbial products related to promotion of disease phenotypes have been identified with such approaches. Functional profiling of the intestinal microbiome needs to be implemented in the field of food allergy.

Mouse models have clearly demonstrated the potential for the microbiota to regulate sensitization to foods. Antibiotic-treated mice, germ-free mice, and those with low-diversity microbiota are susceptible to food allergy (Cahenzli et al., 2013; Stefka et al., 2014). Mice that are genetically susceptible to food allergy have an intestinal microbial signature that is significantly different from resistant wild-type mice, and transfer of the microbiota to germ-free mice transfers the susceptibility (Novak Rivas et al., 2013). Clostridia species within the commensal microbiota have been identified as suppressive to the development of peanut allergy (Stefka et al., 2014). Clostridia species derived from mice or human microbiota have been identified that are regulatory and suppress the development of food allergy when administered therapeutically to mice (Atarashi et al., 2011, 2013).

The mechanisms by which the microbiota may alter food allergy susceptibility are diverse. Unlike other immunoglobulin isotypes, IgE is elevated in the absence of microbiota. Expression of MyD88 by B cells was necessary to limit IgE levels as well as basophil populations in mice (Hill et al., 2012). Germ-free mice have elevated levels of IL-33 expression in the intestinal epithelium (Ohnmacht et al., 2015), which can drive

Table 1. Major Food Allergens and Their Components

Food	Allergenic Components	Family	Molecular Weight (kDa)
Cow's milk	Bos d 9	alphS1-casein	23.6
	Bos d 10	alphaS2-casein	25.2
	Bos d 11	beta-casein	25
	Bos d 12	k-casein	19
	Bos d 4	alpha-lactalbumin	14.2
	Bos d 5	beta-lactoglobulin	18.3
	Bos d 6	bovine serum albumin	67
	Bos d 7	Immunoglobulin	160
	Bos d 8	caseins	20–30
Hen's egg	Gal d 1	ovomucoid	28
	Gal d 2	ovalbumin	44
	Gal d 3	ovotransferrin/conalbumin	78
	Gal d 4	lysozyme	14
	Gal d 5	serum albumin	69
	Gal d 6	YGP42	36
Fish (Atlantic herring, carp, codfish, atlantic cod, tuna, etc.)	Clu h 1, Cyp c 1, Gad c 1, Gad m 1, Lat c 1, Lep w 1, Onc m 1, Ras k 1, Sal s 1, Sar sa 1, Seb m 1, Thu a 1, Xip g 1	parvalbumin	10–12
	Onc k 5	vitellogenin	18
	Sal s 2, Gad m 2, Thu a 2	enolase	47
	Sal s 3, Gad m 3, Thu a 3	aldolase	40
Crustacean shellfish (shrimp, lobster, crab)	Cha f 1, Cra c 1, Por p 1, Hom a 1, Pen s 1, Lit v 1, Pen m 1, Met e 1, Pan b 1, Pen a 1, Pen i 1, Por p 1, Pan s 1	tropomyosin	36–38
	Cra c 2, Lit v 2, Pen m 2	arginine kinase	40–45
	Cra c 5, Lit v 3, Pen m 3, Hom a 3	myosin light chain	17–23
	Cra c 4, Lit v 4, Pen m 4	SCP	25–20
	Cra c 6, Pen m 6, Hom a 6	troponin C	20
	Cra c 8, Arc s 8	triose phosphate isomerase	28
Tree nuts (almond, walnut, hazelnut, cashew, pecans)	Pru du 4, Cor a 2	profilin	14
	Pru du 3, Jug r 3, Cor a 8	non-specific lipid transfer protein	9
	Pru du 5	60 s acidic ribosomal protein	10
	Jug r 1, Car i 1, Ana o 3, Cor a 14	2S albumin	14–17
	Jug r 2, Jug r 6, Car i 2, Ana o 1, Cor a 11	7S globulins (vicilin-like)	40–55 (subunit)
	Pru du 6, Jug r 4, Cor a 9, Car i 4, Ana o 2	11S globulin (legumin-like)	40–60 (subunit)
	Jug r 5, Cor a 1	PR-10, Bet v 1 family member	17–20
	Cor a 12, Cor a 13	oleosin	14–17

(Continued on next page)

Table 1. Continued

Food	Allergenic Components	Family	Molecular Weight (kDa)
Peanut	Ara h 1	cupin, vicillin-type 7S globulin	64
	Ara h 2	conglutin (2S albumin)	17
	Ara h 3	cupin (11S globulin)	60 (subunit)
	Ara h 5	profilin	15
	Ara h 6	conglutin, 2S albumin	15
	Ara h 7	conglutin, 2S albumin	15
	Ara h 8	PR-10, Bet v 1 family member	17
	Ara h 9	lipid transfer protein type 1	9.8
	Ara h 10	oleosin	16
	Ara h 11	oleosin	14
	Ara h 12	defensin	8
	Ara h 13	defensin	8
	Ara h 14	oleosin	17.5
	Ara h 15	oleosin	17
	Ara h 16	lipid transfer protein type 2	8.5
	Ara h 17	lipid transfer protein type 1	11
Wheat ^a	Tri a 14	lipid transfer protein 1	9
	Tri a 18	agglutinin isolectin 1	17
	Tri a 19	pomega 5-gliadin	65
	Tri a 20	gamma-gliadin	35–38
	Tri a 25	thioredoxin	13
	Tri a 26	high-molecular-weight glutenin	88
	Tri a 36	low-molecular-weight glutenin	40
	Tri a 37	alpha purothionin	12
	Tri a 41	mitochondrial ubiquitin ligase activator of NFκB 1	
	Tri a 42	hypothetical protein from cDNA	
	Tri a 43	hypothetical protein from cDNA	11.7
	Tri a 44	endosperm transfer cell specific PR60	12
	Tri a 45	elongation factor 1	9.7
Soy	Gly m 3	profilin	14
	Gly m 4	PR-10, Bet v 1 family member	17
	Gly m 5	beta-conglycinin, 7S globulin	52–76 (subunit)
	Gly m 6	glycinin, 11S globulin	58–69 (subunit)
	Gly m 7	seed biotinylated protein	76.2
	Gly m 8	2S albumin	18.5

Only those allergens in which clinical relevance has been determined (listed as allergens at WHO/IUIS) are included on the list.

Source: allergome (<http://www.allergome.org/>), WHO/IUIS (<http://www.allergen.org/>)

^aWheat allergens: components from both food and respiratory wheat allergy have been listed.

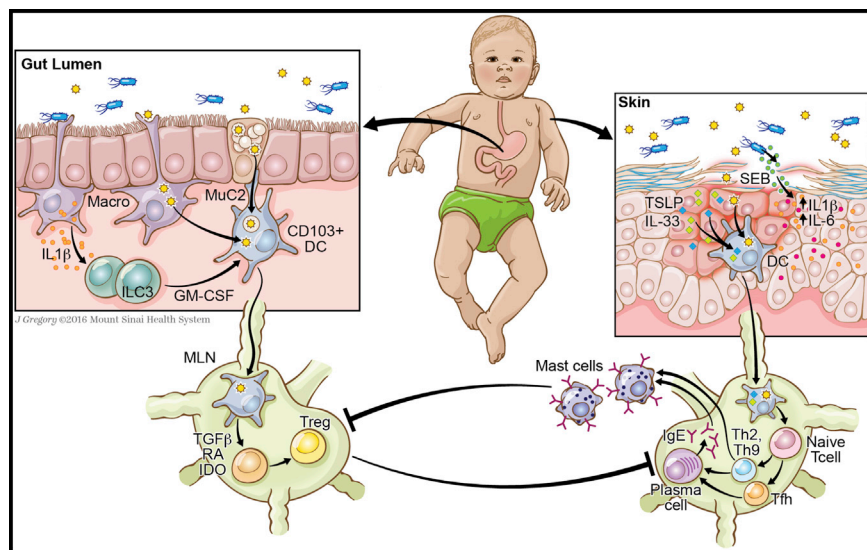


Figure 2. The Dual Exposure Hypothesis of Food Allergy and Tolerance

Exposure to antigens through the oral route results in a tolerance response, involving CD103⁺ DCs that are instructed toward a tolerance phenotype promoted by local factors such as GM-CSF and Muc2. CD103⁺ DCs migrate to the mesenteric lymph nodes to induce Treg cells. By contrast, antigen exposure through eczematous skin (including damage or bacterial toxins) leads toward sensitization. Cytokine release by epithelial cells instructs skin DCs. DCs transport the antigen to the draining lymph nodes where Th2 and Tfh cells are generated. The two pathways (sensitization and tolerance) regulate each other through inhibitory mast cell/Treg cell interactions.

dizygotic twins (64.3% versus 6.8%), suggesting a strong genetic influence of peanut allergy (Sicherer et al., 2000). A genome-wide association study of 2,759 US participants identified variants

Th2 cell-type responses in the intestine. Microbial colonization promotes the expansion of Treg cells (Atarashi et al., 2011, 2013; Faith et al., 2014; Wang et al., 2015b). The microbiota expands a subset of peripherally induced Treg cells in the colon that express the transcription factor RORγt and are regulated similarly to Th17 cells. The absence of this subset of RORγt⁺ Treg cells results in exacerbated Th2 cell-type pathology associated with increased GATA3⁺ T cells and GATA3⁺ Treg cells in the intestine (Ohnmacht et al., 2015).

In addition to promoting a regulatory tone in the intestine, the microbiota has an impact on intestinal barrier function. Microbial colonization promotes IgA production (Stefka et al., 2014) by MyD88 signaling in Treg cells leading to regulation of mucosal follicular T (Tfh) cell responses (Wang et al., 2015b). IgA can contribute to tolerance by antigen exclusion. Colonization of germ-free mice with Clostridia strains also induces IL-22 production (Stefka et al., 2014), a cytokine that protects the integrity of the intestinal epithelial barrier and reduces intestinal permeability to dietary antigens. The intestinal microbiota can interact with other important environmental factors such as the diet to suppress the development of food allergy. Dietary fiber is metabolized by anaerobic bacteria into short chain fatty acids (SCFA), such as butyrate, propionate, and acetate. Feeding mice a high-fiber diet suppressed the development of food allergy. High-fiber diets induced the generation of SCFA and the suppression of food allergy in a microbiota-dependent manner, and administration of SCFA alone could suppress food allergy. Immune changes induced by a high-fiber diet included increased Tfh cells, increased IgA, and increased Treg cells and were dependent on signaling through SCFA receptors GPR43 on epithelial cells and GPR109A on immune cells (Smith et al., 2013; Tan et al., 2016). Readers are referred to Wesemann and Nagler (2016) for more information on the mechanisms by which microbiome regulates susceptibility to allergic disease.

Genetic Risk Factors of Food Allergy

Family history is a strong risk factor for the development of food allergy. In a twin study focused on peanut allergy, monozygotic twins had a higher concordance rate of peanut allergy than

in HLA-DRB1 and HLA-DQB1 as significantly associated with peanut allergy (Hong et al., 2015) but not milk or egg allergy. The strength of this study was the size of the cohort, but food allergy diagnosis was not stringent and may have included those who were sensitized but not clinically reactive. The association of peanut allergy with variants in HLA-DRB1 was replicated in a GWAS using a cohort of 221 Australian infants, including 73 with a gold-standard diagnosis of oral food challenge-proven peanut allergy (Martino et al., 2017). The only other replicated genetic susceptibility loci for peanut allergy is filaggrin, a gene involved in barrier function of the skin (Brown et al., 2011; Venkataraman et al., 2014), which again supports a role of sensitization through the skin to peanut allergens. There are no well-validated genetic susceptibility loci identified for food allergies other than peanut. There is a need for larger cohort studies that are powered to address the genetic basis of food allergies in general, or of food allergies other than peanut. For example, it is not understood whether the paradigm of skin sensitization also applies to common allergens other than peanut.

Treatment of Food Allergy

Antigen-Specific Immunotherapy

Currently there is no FDA-approved treatment for food allergies. The current standard of care is allergen avoidance and rapid administration of rescue medication such as antihistamines or epinephrine in the case of accidental exposure. The most promising treatment under investigation is the use of antigen-specific immunotherapy, which consists of providing increasing amount of allergen over an extended period of time with the aim to suppress food-allergy symptoms. Allergen immunotherapy has been tested by the subcutaneous, oral, sublingual, and epicutaneous routes. Subcutaneous immunotherapy of unmodified food extracts was discontinued due to severe adverse reactions to the therapy. Oral and epicutaneous immunotherapy for peanut are now in Phase III trials, and sublingual immunotherapy is still under active investigation.

Milk and egg allergy are the most common and earliest-encountered food allergies in young infants (National Academies

Table 2. Summary of Randomized, Double-Blind, Placebo-Controlled Studies about Early Introduction of Foods for Prevention of Food Allergy

Study (Trial)	Food Introduced	Dose	Calculated Egg White Protein/Week	Age at Introduction	Age at Oral Challenge	Prevalence (Intent to Treat)	
						Early Introduction	Placebo
Palmer et al., 2013 (STAR)	egg	0.9 g pasteurized raw whole egg powder daily	1.6 g	4 months	12 months	33% (4/42)	51% (18/35)
Du Toit et al., 2015 (LEAP)	peanut	6 g peanut protein per week	–	4–11 months	60 months	3.2% (10/307)	17.2% (54/314)
Du Toit et al., 2016 (LEAP-On)	follow-up of LEAP study (avoidance of peanut for 12 months)	–	–	–	72 months	4.8% (13/270)	18.6% (52/280)
Perkin et al., 2016 (EAT)	peanut, cooked white egg, cow's milk, sesame, white fish, wheat	2 g of allergen protein twice weekly	4 g	3 months	1–3 years	5.6% (32/567) ^a	7.1% (42/595) ^a
Natsume et al., 2017 (PETIT)	heated egg	25 mg egg protein (50 mg heated egg powder) daily for 3 months and 125 mg egg protein (250 mg heated egg) for another 3 months	0.1 g (6–9 months) and 0.5 g (9–12 months)	6 months	12 months	8% (5/60)	38% (23/61)
Bellach et al., 2017 (HEAP)	egg white	0.83 g egg white protein (pasteurized egg white powder) the first week, 1.66 g second week, and 2.5 g from third week, 3 times per week	2.5 g first week, 5 g second week, and 7.5 g from the third week	4–6 months	12 months	2.1% (3/142)	0.6% (1/156)
Palmer et al., 2017 (STEP)	egg	0.9 g pasteurized raw whole egg powder daily	1.6 g	4–6 months	12 months	7% (26/371)	10.3% (39/377)

^aPer protocol: 2.4% (5/208) in early introduction versus 7.3% (38/524) in standard introduction

of Sciences Engineering and Medicine (U.S.), 2016). As noted above, the majority of young milk- and egg-allergic children can tolerate milk and egg in heat-denatured (baked) products, e.g., breads, muffins, cakes, etc. Subsequent studies incorporating baked-milk and/or baked-egg products into the diets of these young children was shown not only to be safe and reduce dietary restrictions but to accelerate the development of full tolerance to all forms of milk and egg (Kim et al., 2011; Leonard et al., 2012). Consequently, this form of “immunotherapy” is becoming a standard practice with clinicians identifying young children who tolerate heat-denatured milk and/or egg products early and incorporating such products into their daily diets (Sampson et al., 2014).

Oral immunotherapy (OIT) is the most studied route of immunotherapy. OIT begins with a very low dose of allergen that is gradually increased over a period of months to reach a maintenance dose in the range of 300 mg to low gram daily doses. There is a time-dependent increase in the frequency of treatment success, which often requires several years to achieve. Treatment success in OIT trials is the ability to tolerate a food challenge. Tolerating a food challenge while on active treatment is referred to as desensitization, while tolerating a food challenge after treatment has been discontinued (typically 1–3 months after discontinuation) has been referred to as sustained unresponsiveness. The term “tolerance” is avoided due to the fact that clinical reactivity frequently recurs in a time-dependent manner. We propose that a more accurate terminology to describe the sustained effect of allergen immunotherapy is “remission.” There have now been many OIT studies, although gold-standard randomized, double-blind, placebo-controlled trials of OIT are few in number and the variability in study design between trials can make comparisons difficult. In general, OIT for milk, egg, and peanut effectively desensitizes the majority of subjects tested. Remission occurs in up to 50% of subjects treated. A recent study of young children aged 9–36 months treated with OIT for peanut allergy showed an overall remission rate of 78%, indicating that initiating treatment early promotes treatment success (Vickery et al., 2017).

Adverse reactions to treatment are common with OIT. Approximately 15%–20% of subjects are unable to tolerate OIT due to persistent gastrointestinal symptoms. In those who can tolerate OIT, there is an elevated risk of systemic reactions often associated with factors such as acute infectious illness, exercise, or menstruation. The use of omalizumab, a monoclonal antibody that binds to IgE and prevents its binding to FcεRI on basophils and mast cells, in combination with OIT has shown increased safety. Administration of omalizumab with OIT allows a more rapid up-dosing of food allergens to reach a maintenance phase (MacGinnitie et al., 2017; Nadeau et al., 2011). In a recent study comparing OIT with or without omalizumab for the treatment of cow’s milk allergy, there was a significant improvement in safety (medians: 2.1% versus 16.1% of doses provoking symptoms, and 0 versus 3.8% of dose-related reactions requiring treatment), while the remission rate was not significantly changed (48.1% in omalizumab versus 35.7% in the placebo group) (Wood et al., 2016). Therefore, addition of omalizumab to OIT retains the efficacy of OIT while improving the safety profile. In Table 3, a list of ongoing food allergy trials is shown.

Sublingual immunotherapy (SLIT) and epicutaneous immunotherapy (EPIT) are administered as drops under the tongue or Viaskin patch, respectively. Doses of antigen administered are substantially lower than that delivered by oral immunotherapy (1–10 mg for SLIT and 100–250 µg for EPIT). The lower dose of administration contributes to a better safety record than OIT, with adverse reactions being primarily local. The increased safety profile is associated with a smaller treatment effect. Studies examining the efficacy of SLIT and EPIT will often determine treatment success as an increase in threshold (for example a 10-fold increase in threshold dose causing reactions) rather than a complete desensitization. SLIT and EPIT have been shown to be effective in increasing the threshold dose of antigen tolerated. Increasing the threshold may be sufficient as a therapeutic outcome if the goal is to protect against accidental reactions rather than to incorporate the food into the diet.

Mechanism of Food Allergen Immunotherapy

Humoral Mechanisms. During allergen immunotherapy, there is a transient rise followed by a decline in allergen-specific IgE and a steady increase in allergen-specific IgG, especially in IgG4. The different IgG isotypes have been hypothesized to block hypersensitivity reactions by competing with IgE for the allergen. In addition, IgG has been proposed to inhibit IgE-mediated activation of effector cells by binding to the inhibitory receptor FcγRIIb (Strait et al., 2006). An early rise in allergen-specific IgG4 measured after 10 months of egg OIT was predictive of egg-allergy remission at 24 months (Jones et al., 2016), although levels of food-specific IgG4 at the end of treatment did not discriminate between those with desensitization and those with remission (Jones et al., 2016). It is possible that the inhibitory capacity of IgGs generated during OIT correlates better with clinical protection than the quantitative levels in sera, as it has been described for other types of antigen-specific immunotherapy (James et al., 2011). Peanut OIT generates a polyclonal expansion of peanut-specific IgG4 as measured by epitope-specific microarray, while the number of epitopes recognized by IgE contracts (Vickery et al., 2013). Studies looking at peanut-specific B cells sorted using a fluorescent Ara h 2 multimer identified an early expansion of Ara h 2-specific memory B cells after only 7 weeks of treatment (Patil et al., 2015). BCR sequencing revealed hypermutated BCRs that also showed evidence of oligoclonality shared between individuals. Thus, there may be a commonly protective IgG4 repertoire. A second study also identified an increased frequency of Ara h 1- and Ara h 2-specific B cells during OIT that were highly mutated (Hoh et al., 2016). Allergen-specific IgG4 but not IgE showed increased somatic mutation during OIT suggesting that the treatment could promote the induction of IgG4 antibodies with higher affinity to block IgE binding. IgG antibodies induced during milk OIT are able to block IgE-mediated basophil and mast cell activation, acting through FcγRIIb (Burton et al., 2014a). Other humoral mechanisms that may contribute to the effect of allergen immunotherapy include the induction of food-specific IgA that can contribute to antigen exclusion (Kulis et al., 2012b; Wright et al., 2016).

T Cell Response to Food Allergen Immunotherapy. Although there are few studies of allergen immunotherapy for food that have performed T cell profiling, OIT also has been associated with a suppression of the allergen-specific Th2 cytokine

Table 3. Ongoing Immunotherapy Clinical Trials to Food Antigens

Trial ^a	Food	Type of Immunotherapy	Intervention	Age (Years)	Phase	Enrollment
NCT02457416	peanut	OIT	peanut flour	5–15	1, 2	77
NCT01324401	peanut	OIT	peanut flour	7–21	1, 2	30
NCT00932828	peanut	OIT	peanut flour (2 different doses)	9–36 months	1, 2	60 ^b
NCT01750879	peanut	OIT	peanut flour	7–55	1	40 ^b
NCT02203799	peanut	OIT	peanut flour	5–16	2	30 ^b
NCT00815035	peanut	OIT	peanut flour	1–6	1	60 ^b
NCT01601522	peanut	OIT	peanut flour	5–10	2	50 ^b
NCT02103270	peanut	OIT	peanut flour (2 different doses)	8–55	–	120
NCT02402231	peanut	OIT	peanut with omalizumab (Xolair)	12–22	2	23
NCT01867671	peanut	OIT	peanut liquid extract and peanut flour	1–4	2	146
NCT02149719	peanut	OIT	boiled peanut	8–16	2	46 ^b
NCT02198664	peanut	OIT	peanut protein capsule (CPNA)	4–26	2	53 ^b
NCT02198664	peanut	OIT	peanut protein capsule (CPNA)	4–26	2	53 ^b
NCT02635776, NCT02993107 (follow up)	peanut	OIT	AR101 powder	4–55	3	500 ^b
NCT02640014	milk	OIT	milk	>6	–	250 ^b
NCT02465905	cow's milk	OIT	raw milk versus heated milk	3–18	–	50 ^b
NCT02738060	cow's milk	OIT	baked milk products (muffin, pizza cheese)	6 months– 18 years	2	80 ^b
NCT01846208	egg	OIT	egg versus baked egg	3–16	2	92
NCT01822353	egg, milk, nut allergy	OIT	milk, egg protein, nut cream	18–50	2	100 ^b
NCT01980992	wheat	OIT	vital wheat gluten	4–30	1, 2	46
NCT02786914	tree nut or sesame	OIT	tree nut, sesame	4–45	–	250 ^b
NCT01546753	walnut	OIT	walnut protein powder	6–45	1, 2	15 ^b
NCT02626611	multi-food	OIT	food flour (low or high dose) with omalizumab (Xolair)	4–55	2	70 ^b
NCT02643862	multi-food	OIT	food flour with or without omalizumab (Xolair)	4–55	1, 2	60 ^b
NCT02879006	multi-food	OIT	food allergen with omalizumab (Xolair) with or without Chinese herbal medication	6–40	2	34 ^b
NCT01904604	peanut	EPIT	peanut low and high dose Viaskin patch	4–25	2	75
NCT02636699	peanut	EPIT	Viaskin peanut high dose (250 µg)	4–11	3	356
NCT02223182	cow's milk	EPIT	Viaskin milk 3 different doses	2–17	1,2	198
NCT01373242	peanut	SLIT	liquid peanut extract	1–11	1,2	49
NCT02304991	peanut	SLIT	liquid peanut extract	1–4	2	50 ^b
NCT02382718	fish	SCIT	modified fish parvalbumin mCyp c 1	18–65	2	45
NCT02991885	peanut	SCIT	HAL-MPE1 (aluminum hydroxide adsorbed peanut extract)	5–50	1	42 ^b
NCT01431859	fruits involved in oral allergy syndrome (cross-reactivity with birch pollen)	SCIT	Allergovit birch pollen	>18	4	40 ^b

Abbreviations are as follows: OIT, oral immunotherapy; EPIT, epicutaneous immunotherapy; SLIT, sublingual immunotherapy; SCIT, subcutaneous immunotherapy.

^aFrom <https://clinicaltrials.gov>

^bEstimated

response (Gorelik et al., 2015; Varshney et al., 2011). T cell responses have been described as anergic following OIT. In a study of patients undergoing OIT with omalizumab for milk allergy, a reduction of the T cell proliferative response was

observed that could be partially restored with exogenous IL-2 (Bedoret et al., 2012). No evidence of increased milk-responsive Treg cells or IL-10- or TGF- β -dependent suppression was observed in association with OIT. A recent study performed

transcriptional analysis at the single-cell level using Ara h 2-specific dextramers to purify peanut-specific T cells before and after OIT for peanut allergy (Ryan et al., 2016). In subjects with OIT-induced remission, there was an expansion of peanut-specific T cells with a phenotype of low expression of the costimulatory molecule CD28, the proliferation marker Ki-67, and the activation marker CD69. Anergy is a reversible process and may provide an explanation for the lack of duration of OIT-induced remission. Due to the fact that IL-4 contributes to the maintenance of Th2 cell responses and that memory B cells may require IL-4 from T cells to maintain high IgE levels (Jiménez-Saiz et al., 2017), reduction of Th2 cytokine responses during immunotherapy could lead to the decrease of antigen-specific IgE.

Mouse models of OIT or EPIT have identified a critical role for Treg cells, including Foxp3⁺ and LAP⁺Foxp3[−] Treg cells in the clinical protection induced by immunotherapy. Mice treated with EPIT are protected from anaphylaxis by LAP⁺CD4⁺Foxp3[−] T cells that directly suppress mast cell activation by a TGFβ²-dependent mechanism (Tordesillas et al., 2017). There is also evidence from mice that route of allergen immunotherapy can influence the phenotype, homing characteristics of Treg cells, and mechanism of suppression (Dioszeghy et al., 2016). In a model of mouse OIT, transfer of Foxp3⁺ Treg cells from a successfully treated mouse could suppress the development of peanut allergy in the recipient mouse (Burton et al., 2014a). In similar experiments, adoptive transfer of Treg cells from EPIT-treated milk-allergic mice prevented sensitization to peanut and peanut-induced anaphylaxis in recipient mice (Mondoulet et al., 2015).

Based on experimental data in mice and data from other forms of allergen immunotherapy, it has been proposed that humoral responses and direct modulation of basophil signaling are responsible for desensitization, while remission is associated with the development of Treg cells. Currently there are little data from human studies to support that paradigm. As outlined above, several studies have reported negative data for a role of antigen-specific (tetramer-positive) or antigen-responsive (proliferating) Treg cells in the response to OIT (Bedoret et al., 2012). In contrast with those studies, Syed et al. (2014) reported that an increase of antigen-induced Foxp3⁺ T cells that expressed memory markers and produced IL-10 were found in individuals that developed remission in response to OIT for peanut allergy. These peanut-responsive T cells exhibited decreased methylation of CpG sites at the Foxp3 locus in antigen-specific Treg cells, which was found to be reversed in individuals who eventually relapsed after peanut OIT. Limitations of studies to date include small sample size and a reliance on studying immune cells in peripheral blood, which may not reflect events in mucosal sites. Studies using larger cohorts of subjects in randomized, placebo-controlled trials are needed to more conclusively identify the role of T cell anergy, deletion, regulation, or reprogramming in OIT-induced remission.

DCs and Food Allergen Immunotherapy. DCs from patients with food allergy exhibit an altered cytokine response when stimulated with allergen (Frischmeyer-Guerrero et al., 2011). This is likely due to signaling by allergen through IgE bound to FcεRI that is expressed on the surface of human DCs. DCs from allergic subjects treated with OIT or SLIT demonstrate an altered phenotype and responsiveness to innate stimuli as well as a less

mature phenotype after co-culture with CD4⁺ T cells and stimulation with allergen (Frischmeyer-Guerrero et al., 2014; Gorelik et al., 2015). Peripheral blood DCs obtained from subjects after peanut OIT, but not at baseline, suppressed methylation of Foxp3 in T effector cells (Syed et al., 2014). It is known that reduction of circulating IgE levels with omalizumab reduces the expression of IgE on peripheral blood DCs (Prussin et al., 2003), which would also be expected to occur after allergen immunotherapy. This may contribute to reduced IgE-facilitated priming of DCs, although there are conflicting data on the impact of omalizumab on T cell priming by DCs (Foster et al., 2011; Schroeder et al., 2010).

Emerging Food Allergy Therapeutics

Major efforts are being invested to improve the safety and efficacy of allergen immunotherapy for the treatment of food allergy. Improvements in efficacy are primarily focused on additional immunomodulation, while improvements in safety are focused on modified or encapsulated allergens and targeting IgE.

Immunomodulation. The addition of adjuvants that modulate dendritic cell responses away from promoting Th2 cell immunity has been investigated. The addition of CpG motifs that act on TLR9 to allergen immunotherapy can effectively suppress food-induced anaphylaxis in mice (Kulis et al., 2013; Pali-Schöll et al., 2013; San Román et al., 2009). These have been administered either as a soluble mixture of peanut plus oligodeoxynucleotide given by subcutaneous injection (Kulis et al., 2013) or by CpG-coated PLGA nanoparticles administered by the oral route (Srivastava et al., 2016). A previous approach had used *E. coli* producing Ara h 1, Ara h 2, and Ara h 3 that were modified to reduce IgE binding. The *E. coli* would theoretically provide both encapsulation and toll-like receptor (TLR) ligands for immunomodulation. This approach was very effective at suppressing anaphylaxis in a mouse model of peanut anaphylaxis (Li et al., 2003). This approach has been translated into a phase I trial, in which peanut-allergic patients were administered the therapeutic, EMP-123, by the intrarectal route as was effective in mice. Despite the encapsulation, the IgE modification, and the administration by a mucosal route, an unacceptable rate of adverse systemic reactions to the therapy was noted (Wood et al., 2013). Other immunomodulatory strategies such as the use of chemically modified peanut proteins or DNA vaccines codifying for Ara h 1, Ara h 2, and Ara h 3 are currently being evaluated (<https://clinicaltrials.gov>). Peanut allergens coupled with syngeneic splenic leukocytes are moderately effective in reducing peanut-induced anaphylaxis in mice through a Treg-cell-dependent mechanism (Smarr et al., 2011). The syngeneic splenic leukocytes are thought to provide a signal of self that acts as a tolerogenic adjuvant. Another strategy that has been explored to modulate immunotherapy treatment is the fusion of the major peanut allergen Ara h 2 and human IgG Fcγ1 (Liu et al., 2013). This construct inhibited peanut extract-induced allergic reactions by cross-linking inhibitory receptor FcγRIIb with peanut-specific IgE bound to FcεRI.

Microbial Therapeutics. There is clear evidence that the commensal microbiota modulates susceptibility to food allergy, and there is great interest in application of microbial-based therapeutics for the prevention or treatment of food allergy. Studies to date have primarily focused on traditional probiotics. A randomized placebo-controlled trial of peanut OIT administered

with *Lactobacillus rhamnosus* induced remission in 82.1% of treated subjects versus 3.6% in the placebo (untreated) group (Tang et al., 2015). The remission rate is higher than that reported in other studies of peanut OIT, but no comparison arm with peanut OIT alone was performed. Consequently, further investigation will be necessary to determine the contribution of probiotics to the treatment response. The use of oral encapsulated fecal microbiota transplant for the treatment of peanut allergy is currently being evaluated in a phase I clinical trial (ClinVar: NCT02960074). In addition, therapeutic administration of Clostridia strains has been used in mouse models of food allergy, but no translation to human trials has yet been performed.

IgE Inhibitors. The anti-IgE molecule omalizumab is currently being investigated in clinical trials in combination with oral immunotherapy to reduce side effects and duration of treatment. Ligelizumab, an anti-IgE antibody with higher affinity, has been developed and tested in comparison with omalizumab in asthmatic subjects (Gauvreau et al., 2016). Ligelizumab was more effective than omalizumab in suppressing skin prick test responses and had a larger but not statistically significant shift in responses to airway allergen challenge as compared to omalizumab. This monoclonal antibody has not yet been tested in food allergy. Another monoclonal antibody targets a region of membrane IgE on IgE-switched B cells that is not present in serum IgE (M1' domain) (Brightbill et al., 2010). This antibody, developed under the name quilizumab, suppressed allergen-induced challenge increases in specific IgE in asthmatic subjects and reduced early and late phase responses to allergen challenge (Gauvreau et al., 2014). However, it was not found to be clinically effective in the treatment of inadequately controlled allergic asthma (Harris et al., 2016), diminishing the enthusiasm for translating this to food allergy.

Another IgE inhibitor called DARPin E2_79 was described not only to neutralize free IgE but also to displace FcεRI-bound IgE from the surface of human basophils and abrogate IgE-dependent cell activation (Eggel et al., 2014; Kim et al., 2012). A biparatopic DARPin bi53_79, created by fusion of E2_79 and the non-disruptive anti-IgE DARPin E3_53 that recognized receptor-bound IgE, presented even further increased efficacy of disruption of IgE:FcεRI complexes than DARPin E2_79 alone (Eggel et al., 2014). Due to the slow dissociation rate of IgE:FcεRI complexes, the use of this IgE inhibitor could have a greater effect than omalizumab for the treatment of allergic patients.

Small-Molecule Inhibitors. The use of oral rapamycin to inhibit mTOR signaling pathway was effective in suppressing established food allergy in a mouse model of ovalbumin-induced anaphylaxis (Yamaki and Yoshino, 2012a), suppressing mast cell activation and Th2 cytokines. Rapamycin suppresses the proliferation of highly differentiated pathogenic Th2 cells (Yin et al., 2017). The encapsulation of rapamycin with antigen in nanoparticles has been shown to increase the duration of the immunosuppressive effects of rapamycin (Maldonado et al., 2015). Treatment with the receptor tyrosine kinase inhibitor Sunitinib also suppresses systemic and oral induced anaphylaxis by preventing mast cell accumulation and activation in the intestine (Yamaki and Yoshino, 2012b).

Hypoallergenic Mutants and Peptides. Two approaches to increasing the safety of allergen for immunotherapy have been utilized. Recombinant allergens with modified IgE-binding epi-

topes retain the ability to stimulate T cell proliferation but with reduced risk to cause anaphylactic symptoms (Bolhaar et al., 2005; Ferreira et al., 1998; Roulias et al., 2014; Tordesillas et al., 2011; Zuidmeer-Jonghejan et al., 2015). In addition to site-directed mutagenesis, allergen treatments such as pepsin digestion or heat denaturalization resulted in hypoallergenic forms that were effective in reducing allergic reactions in cashew-sensitized mice after an immunotherapy protocol (Kulis et al., 2012a). Peptide-based immunotherapy using peptides corresponding to T cell epitopes also has been explored to suppress allergic responses. Therapeutic treatment of OVA-sensitized mice with three immunodominant T cell epitopes of hen ovalbumin suppressed hypersensitivity reactions and IgE levels (Yang et al., 2010). Similarly, treatment with a T cell epitope peptide mixture was effective in reducing allergic response in a model of shrimp allergy (Wai et al., 2016). Despite the lack of IgE binding of these modified antigens, side effects may occur due to late-phase adverse reactions caused by the presence of T cell epitopes (Haselden et al., 1999; Valenta et al., 2016).

Traditional Chinese Medicine. An allergen non-specific approach to treatment has been the development of herbal formulas with immunomodulatory properties. Oral treatment of peanut-allergic mice with the Food Allergy Herbal Formula-2 (FAHF-2), a Chinese herbal medication compound of nine extracts, completely prevented peanut-induced anaphylaxis for more than 36 weeks after discontinuing the treatment (Srivastava et al., 2009). FAHF-2 has immunomodulatory effects on T cells, IgE, and mast cells and basophils (Song et al., 2010; Srivastava et al., 2009). In a phase I trial, FAHF-2 was well tolerated and safe (Patil et al., 2011). In a placebo-controlled trial for safety and efficacy, subjects with food allergies (to peanuts, tree nuts, sesame, fish, or shellfish) were treated for 6 months with FAHF-2, followed by a double-blind placebo-controlled food challenge (Wang et al., 2015a). There was no significant clinical or immunological effect of FAHF-2 treatment. Poor adherence to the treatment was noted due to dosing issues (Wang et al., 2015a). The identification of active compounds (Yang et al., 2014) may allow for further refinement of the formula.

Summary and Future Directions in Food Allergy Research

Food allergy research has made great strides in the last 10 years. Clinical trials have led to changes in practice regarding early feeding guidelines to prevent food allergy and the incorporation of foods such as baked egg or milk into the diet to hasten food allergy resolution. These simple evidence-based practices will have far-reaching impact improving the lives of millions worldwide. Two allergen immunotherapy approaches are now in phase III trials for the treatment of peanut allergy. Basic science studies have identified immune pathways beyond conventional Th2 cell immunity in food allergy pathogenesis, including a network involving IL-9, mast cells, and regulatory T cells. There are key areas that require greater research focus. Despite the central role of IgE in food allergy, the regulation of IgE production by B cells is very poorly understood. Understanding the regulation of allergen-specific IgE at the cellular level is key to designing therapeutics that will eliminate production of IgE. Better-designed studies examining early-life microbiome in

human cohorts are needed to understand the functional implications of dysbiosis leading to food allergy in order to bridge the gap between our understanding of dysbiosis and the development of effective microbial therapeutics.

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H.A.S. is a part-time employee of DBV Technologies and has served on a Scientific Advisory Board for N-Fold LLC, UCB S.A., and Hycor.

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