

STATE OF THE ART REVIEWS ON MECHANISMS OF ALLERGIC DISEASE

Pathogenesis of IgE-mediated food allergy

M. C. Berin

Pediatric Allergy and Immunology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Clinical & Experimental Allergy

Correspondence:

Cecilia Berin, Pediatric Allergy and Immunology, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1198, New York, NY 10029, USA.

E-mail: cecilia.berin@mssm.edu

Cite this as: M. C. Berin, *Clinical & Experimental Allergy*, 2015 (45) 1483–1496.

Summary

Food allergy is a prevalent disease for which there is no current treatment beyond careful food avoidance. Accidental exposure to foods causes reactions in allergic individuals that can range in severity from mild skin reactions to severe and life-threatening anaphylaxis, and there are no validated tools to predict severity of reactions. A greater understanding of the pathogenesis of food allergy is needed to develop prevention and treatment strategies for food allergy. In the last few years, there have been significant developments in the field of food allergy that have led to new ideas about food allergy prevention, diagnosis, and treatment. This review will discuss these recent advances in the food allergy field as well as identify gaps in our knowledge about the immune mechanisms of allergy and tolerance to foods.

Introduction

Food allergy is defined as ‘an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food’ [1]. Within this definition are (1) IgE-mediated food allergies including food-induced anaphylaxis, immediate gastrointestinal hypersensitivity, acute urticaria, and oral allergy syndrome, as well as (2) non-IgE-mediated food allergies including food protein-induced enterocolitis syndrome (FPIES) and (3) eosinophilic gastrointestinal disorders (EGID). This review will focus on the pathogenesis of IgE-mediated food allergy, and readers are referred to recent reviews on the pathogenesis of FPIES [2] and EGID [3]. Recent findings from clinical and translational studies, as well as animal models that shed light on immune mechanisms responsible for the development of tolerance or allergy to foods, will be discussed.

Immune profile of food allergy in humans

Food-specific antibodies

IgE-mediated food allergy is by definition associated with the presence of allergen-specific IgE antibodies, which are measured in serum or inferred by a positive

skin prick test (SPT) to allergen. Sensitization does not equal clinical allergy, and presence of IgE by either SPT or measurement in serum is not diagnostic. Thresholds have been identified for levels of peanut, milk, and egg-specific IgE measured by ImmunoCAP that are 95% predictive of clinical reactivity [4] although there have been differences in 95% predictive threshold values between different study populations [5, 6] and across different age groups [7]. Furthermore, threshold values identified using clinic populations have been shown to have limited predictive value when applied to general populations [8]. Diagnosis of allergy to some foods, such as peanut, is enhanced by measurement of IgE to specific allergens or components. For example, IgE against the major peanut allergen Ara h 2 has greater specificity for peanut allergy than peanut-IgE [9]. In contrast, mono-sensitization to Ara h 8, a homolog to the birch pollen allergen Bet v 1, is associated with tolerance to peanut [10]. IgE antibodies differ in affinity, a measure that is not reflected by standard quantification of IgE. In addition, the polyclonality of the antibody response will influence the likelihood that IgE will be cross-linked by allergen. Using a peptide microarray approach, the diversity of epitope recognition within the major peanut allergens Ara h 1, Ara h 2, and Ara h 3 was found to correlate weakly with peanut-specific IgE levels, but serum with high epitope diversity

induced greater levels of basophil activation than serum with low epitope diversity [11]. Increased epitope diversity within an allergen increases the probability that the allergen will be recognized by two IgE molecules on the effector cell, leading to cross-linking and degranulation. Peptide microarray technology has been used to assess both epitope diversity and affinity of the IgE response to foods, and both of these measures are associated with clinical reactivity [11, 12]. Peptide microarray is currently a time-intensive research approach, and there is a need to translate this into a high-throughput standardized assay and to compare sensitivity and specificity against diagnostic gold standards (double-blind placebo-controlled food challenge) before its diagnostic potential can be realized. In addition to affinity and polyclonality, Shade et al. [13] recently demonstrated that glycosylation of IgE at a single residue in the constant domain 3 is essential for maintaining appropriate structure of IgE, allowing binding to FcεRI and activation of mast cells. It is not known whether there are natural variations in the glycosylation at this site (asparagine-394) that could contribute to differential capacity of IgE to activate allergic effector cells. Carbohydrate-dependent binding of IgE to galectin-3 (formerly known as IgE-binding protein) was shown to vary between different donors, with a subset of the bulk polyclonal IgE within each individual demonstrating binding [14]. Thus, there may be varying proportions of antigen-specific IgE with biological activity.

Skin prick tests are a functional measure of the allergen-specific IgE-mast cell unit that as a bioassay would be expected to be more informative than measurement of allergen-specific IgE in serum. However, SPT responses have to date not been found to be much more predictive than serum levels of specific IgE [15]. Basophil activation tests (BAT) have been used in research to monitor responses to allergen immunotherapy and more recently have been shown to have utility in predicting clinical reactivity to peanut. Santos et al. [16] showed in a cohort of 104 children undergoing oral food challenge (OFC) to peanut that BAT had a significantly better predictive value compared to other tests including peanut-IgE, Ara h 2-IgE, and SPT. This was particularly true when using a subset of patients with inconclusive peanut-IgE and SPT results, leading the authors to propose a stepwise approach of using standard IgE or SPT results as a first step, followed by BAT. Another interesting development was the recent finding by the same authors that BATs were predictive of severity of reactions and allergen threshold dose upon OFC [17]. However, it was previously reported that parameters including BAT were not significant predictors of severity of reactions to peanut [18], although a number of immunologic parameters were significantly correlated with threshold dose-triggering symptoms. Both threshold dose and reaction severity

are measures that could be of major interest to patients in understanding the risk of reactions. Because of these conflicting results, further studies are needed to assess the true predictive capacity of BAT for severity of reactions to peanut, to determine whether the BAT has predictive value for other common food allergies, and to develop these tests for use outside of the research laboratory. BAT does not take into account important factors such as resistance to digestion or absorption across the gastrointestinal epithelium that may contribute to severity of reactions, and also may be variable within individuals over time.

Antibody isotypes other than IgE do not discriminate between allergic and tolerant individuals. Although a food-specific antibody response is often comprised of multiple isotypes including IgA and IgG, the presence of food-specific IgG or IgA is not indicative of inappropriate immune reactivity and can be found in healthy individuals. IgG4 and IgA responses to foods are elevated in response to allergen immunotherapy for the treatment of food allergy and correlate with clinical protection [19, 20]. There are also reports that IgE/IgG4 ratios may be more predictive of clinical reactivity to extensively heated forms of egg than IgE levels alone [21, 22], suggesting a protective role for IgG4 antibodies in homeostatic tolerance to foods.

Food allergen-specific T-cell responses

Food allergy, like other allergic diseases, is associated with a Th2-skewed allergen-specific T-cell profile. This has been demonstrated with increasing levels of technical sophistication, starting from initial observations with allergen-specific T-cell lines, to the identification of proliferating cells after days in culture with allergen [23], to the identification of allergen-specific T cells by MHC Class II tetramers containing Ara h 1 epitopes [24]. T-cell responses to peanut, egg, and milk are associated with a Th2 profile in allergic individuals [25, 26]. T cells producing IL-9 have also been recently described as increased in individuals with peanut allergy, and to be produced by cells distinct from those producing other Th2 cytokines including IL-5 [27]. The frequency of peanut-specific T cells is at least tenfold less in healthy compared to allergic individuals [24, 25]. Although the food allergen-specific response of healthy individuals has been described as Th1, the frequency of IFN-γ-producing T cells is not higher in healthy compared to allergic individuals [25]. The critical difference between food allergic individuals and healthy controls is the presence of Th2 or Th9 cells in allergic individuals. Th2 cells contribute to food allergy through promotion of IgE class-switch by B cells. IL-9 is a growth factor for T cells and can enhance mucin production from epithelial cells, but is perhaps best known as a

factor driving tissue recruitment and activation of mast cells. Therefore, together these allergen-specific Th2/Th9 cells promote development of the effector arm (the components of the immediate hypersensitivity reaction) of food allergy. Although there are not many studies quantifying allergen-specific regulatory T cells, the frequency of these cells has not been shown to be different in allergic vs. tolerant controls [28]. However, resolution of milk allergy has been shown to be associated with an increase in allergen-responsive Tregs [29].

Dendritic cells

Food allergens such as peanut and tree nut allergens have stimulatory effects on innate immune cells, including dendritic cells (DCs) [30–32]. These allergens can activate cells through pattern recognition receptors such as DC-SIGN, and change the phenotype of the DC leading to Th2 cytokine production in responder T cells. In addition to these innate responses, human DCs express the high-affinity IgE receptor FcεRI, and can acquire allergen for presentation through IgE-facilitated uptake [33]. This mechanism of uptake alters the phenotype of the DC, again promoting a Th2-skewed response from T cells [34]. Therefore, once sensitization has occurred, presentation via IgE-facilitated uptake may reinforce the Th2-biased immune response to that food. Reduction of serum IgE with omalizumab (anti-IgE antibody) reduces IgE on the surface of DCs and alters facilitated presentation to T cells [35]. Allergen immunotherapy is associated with changes at the level of the DC. DCs obtained post-immunotherapy induce less Th2 response than DCs obtained pre-immunotherapy [36], and promote the development of Tregs [37]. These results highlight the central role of DCs in programming the T-cell response to foods.

Immune mechanisms of allergic reactions to foods

Type I hypersensitivity responses triggered by cross-linking of IgE bound to the surface of mast cells and basophils underlie the adverse reactions to foods in IgE-mediated food allergy. In mice, depletion experiments have shown that mast cells are the primary cells that contribute to peanut-induced anaphylaxis [38, 39], although minor contributions from mast cell-independent pathways have also been demonstrated [39, 40]. Depletion of basophils has a relatively minor effect on anaphylaxis severity in mice [39, 40], while anaphylaxis is abolished in the absence of mast cells. Studies in human subjects treated with omalizumab (anti-IgE) have suggested a role for basophils in food allergy. Omalizumab induces more rapid suppression of basophils than mast cells, and prevents symptoms during a peanut OFC prior to having a significant suppressive

effect on mast cells as measured by skin prick testing [41]. This was used to argue for a role of basophils in food-induced reactions. However, mast cells are a heterogeneous population of cells, and it is possible that mucosal mast cells were effectively suppressed by omalizumab prior to cutaneous mast cells. To more conclusively test the role of mast cells and basophils in anaphylaxis, better markers of cell type-specific activation are needed so that *in vivo* activation of cells during allergen challenges can be monitored. Histamine and platelet-activating factor (PAF) are the main mediators of anaphylaxis in mice [42] and are also elevated in patients presenting in the emergency room with anaphylaxis [43]. Antihistamines are a standard first-line treatment for mild reactions to foods, demonstrating the role of this pathway. Low levels of PAF acetylhydrolase, the enzyme that inactivates PAF, were found in patients with fatal peanut-induced anaphylaxis [44]. IgG-mediated anaphylaxis has also been demonstrated in mice [45]. Myeloid cells are activated by allergen–IgG complexes and can trigger anaphylaxis in mice through the release of PAF. Under more physiologic conditions (either oral or low-dose systemic allergen challenge), IgE-mediated pathways predominate in mice [40, 46]. IgG-mediated activation of neutrophils *in vitro* has been demonstrated using human blood [47]. There is no evidence at this time that IgG-triggered immune activation contributes to food allergy in humans although this has not yet been carefully studied.

An assessment of symptoms upon challenge identifies the skin as the most common site of food allergy manifestations (hives in > 90% of OFC), followed by the gastrointestinal tract (vomiting, diarrhoea, abdominal pain) and the respiratory tract (wheezing, coughing) [48]. Challenge with peanut caused respiratory reactions more commonly than other food allergens, while peanut and egg were the most common triggers of gastrointestinal symptoms. The immune mechanisms responsible for these organ-specific manifestations of food allergy are not understood. It is surprising that gastrointestinal manifestations are not more common given that it is the first site of contact with the food allergen. Allergens that are readily absorbed across the gastrointestinal tract in an intact form are more likely to trigger systemic reactions in mice [49]. The food matrix, heating, processing, and fat content are factors that can influence the development of reactions to foods by altering the uptake of the foods across the mucosal barrier [50, 51].

Mechanisms of sensitization to foods

Sensitization to foods is an abnormal response to exposure to innocuous antigens, and we do not yet have a good understanding of why sensitization occurs in

some individuals. Genetics plays an important role, similar to other atopic disorders. But the rapid increase in prevalence of food allergy among children suggests that there may be important environmental or lifestyle factors playing a role in susceptibility to food allergy. Changes in diet and our microbial environment, as well as other environmental factors, may all contribute to this increasing prevalence of food allergy. The evidence for each of these factors is outlined below.

Oral tolerance

The default response to antigens delivered to the gastrointestinal tract is active immune tolerance, as first described by Wells and Osborne in 1911 [52]. Oral tolerance is defined as systemic immune non-responsiveness to antigens first encountered by the oral route. Although oral tolerance is defined experimentally (as a suppressed response to immunization following antigen feeding), it has also been demonstrated to occur in humans [53]. Oral tolerance is an active immune response that can be transferred to a naïve animal through the transfer of regulatory T cells. Feeding of antigen generates antigen-specific regulatory T cells, including Foxp3⁺ Tregs [54, 55] as well as latency-associated peptide (LAP)⁺Foxp3⁻ Tregs commonly referred to as Th3 cells [56]. Both of these Treg subsets suppress through TGFβ-dependent pathways [54, 55]. Regulatory T cells generated in the presence of IL-10, and characterized as producing high levels of IL-10, low levels of IL-2, and no IL-4, were termed Tr1 cells and shown to suppress the development of colitis induced by naïve T-cell transfer into severe combined immunodeficiency (SCID) mice [57]. Tr1 cells are important for the suppression of inappropriate immune responses to the commensal microbiota, but have not been shown to play an important role in tolerance to foods [58].

Tregs are generated in the gastrointestinal tract by a specialized population of DCs that acquire antigen in the lamina propria and deliver it to the mesenteric lymph nodes (MLN) [59]. CD103⁺ DCs in the MLN of mice and humans preferentially induce Tregs through the production of TGFβ, retinoic acid (RA), and the enzyme IDO [60–63]. Although a subset of mononuclear phagocytes can reach through the epithelium of the lamina propria to capture antigens directly from the lumen, these cells are transcriptionally similar to macrophages and do not migrate in the steady state [64, 65]. They do, however, transfer antigen to CD103⁺ DCs that then migrate to the MLN [66], demonstrating a division of labour between antigen sampling and presentation. Antigen can also pass through goblet cells via 'goblet cell-associated antigen passages', after which they are taken up by CD103⁺ DCs [67]. The passage of antigens through goblet cells is particularly interesting as the intestinal mucin MUC2, a

goblet cell-derived mucin protein, was recently shown to promote the development of oral tolerance [68]. MUC2 binds to CD103⁺ DCs via a receptor composed of galectin-3, FcγRIIb, and Dectin-1 and drives expression of TGFβ, RA, and IL-10 [68], factors critical for the induction of iTregs in the MLN. Mice lacking MUC2 were impaired in the development of oral tolerance, while exogenous delivery of mucin with antigen restored the development of tolerance in MUC2-knockout mice. Intestinal factors driving tolerance also include Csf2 (GM-CSF), which is produced by innate lymphoid cells (ILC3 cells) of the gastrointestinal tract [69]. Intestinal macrophages produce IL-1β in response to signals from the commensal microbiota, which in turn drives Csf2 expression by ILC3 cells. Csf2, such as MUC2, promotes the development of oral tolerance by driving a regulatory phenotype of CD103⁺ DCs, and by that mechanism enhances the development of iTregs. In summary, under steady-state conditions, the mucosal immune system is primed to generate regulatory responses to antigens in the absence of pathogenic signals that can function as adjuvants. In mice, the default response to fed antigens is tolerance, and adjuvants such as cholera toxin (CT) or staphylococcal enterotoxin B (SEB) are commonly used to break tolerance and generate sensitization [70, 71]. Translating this to human disease has led to two hypotheses. The first is that oral exposure is inherently tolerizing and that sensitization to foods occurs because of exposure to potent allergens through other non-tolerogenic routes. This hypothesis is depicted in Fig. 1. The second is that exogenous factors suppress the tolerogenic environment of the gut (by directly altering the mucosal immune system or indirectly through modulation of the intestinal microbiota), leading to sensitization.

Site of sensitization

Skin exposure has been proposed to be a driving factor in sensitization to foods, particularly in sensitization to peanut. It has long been recognized that there is a unique communication between the skin and the gastrointestinal tract in the context of food allergy. The most common manifestations of food allergy affect the skin, leading to acute hives or exacerbating eczema. Eczema in early life is also a significant risk factor for the development of food allergy [72]. In 2003, it was reported that the use of skin creams containing peanut oil in infants with a history of eczema was significantly associated with peanut allergy (with an odds ratio of 6.8) [73]. The use of wheat-containing soaps has also been identified as a risk for wheat allergy [74]. Increased household consumption of peanut was found to be a risk factor for peanut allergy [75]. Maternal consumption of peanut during pregnancy and breastfeeding was significantly associated with peanut allergy

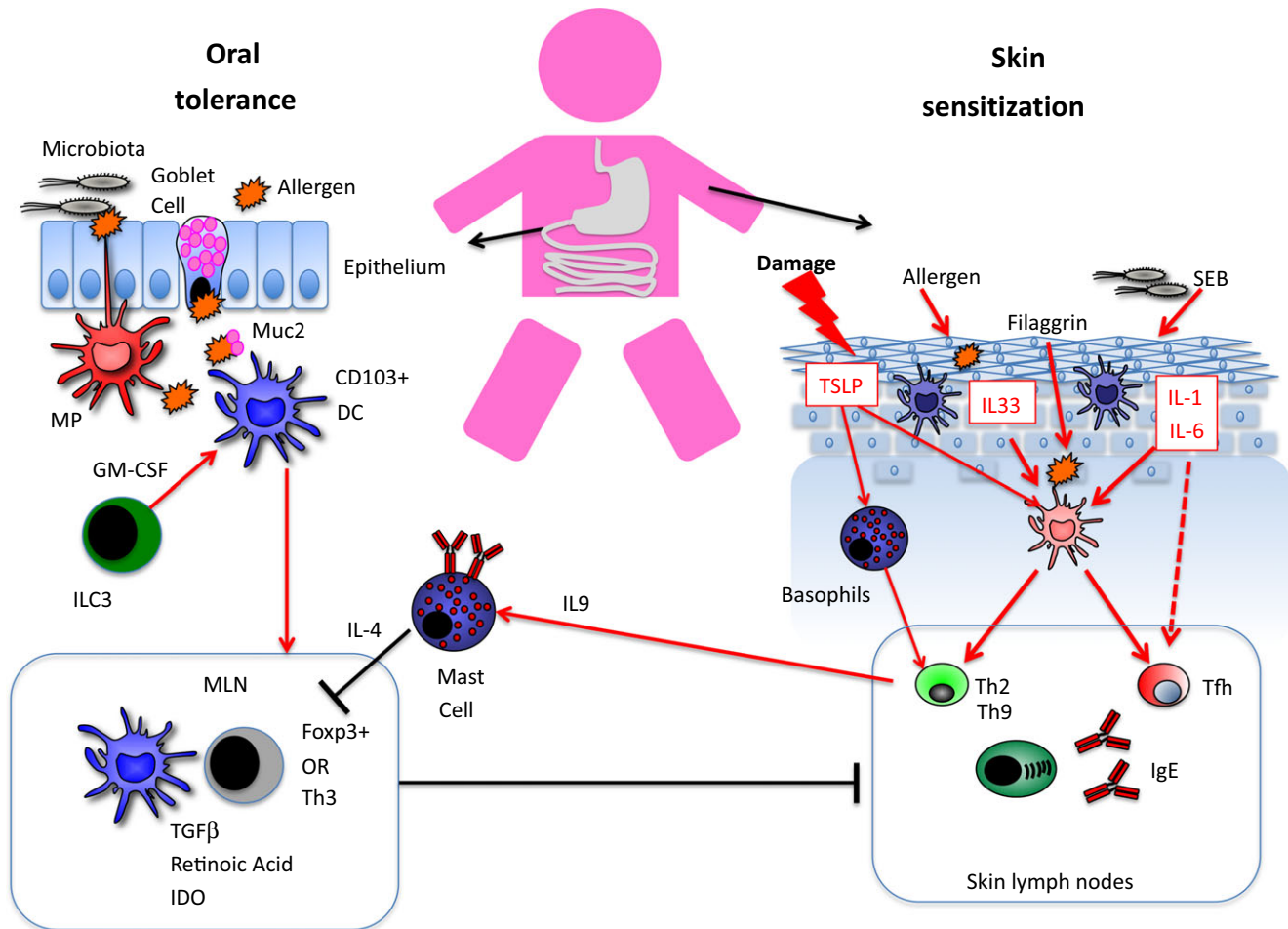


Fig. 1. Immune mechanisms of tolerance and sensitization to foods. The default immune response to oral exposure to foods is tolerance. GM-CSF produced by innate lymphoid cells (ILC3) and Muc2 produced by goblet cells acts on CD103⁺ DCs to promote a tolerogenic phenotype (increased expression of TGF β and retinoic acid). CD103⁺ DCs acquire antigen via goblet cells or mononuclear phagocytes (MP) that extend dendrites into the intestinal lumen. DCs traffic to the mesenteric lymph node (MLN) and generate Tregs, that can suppress sensitization throughout the body. Exposure to food antigens on the skin in the presence of damage, decreased barrier function (filaggrin mutations) or the bacterial toxin SEB drive sensitization by acting on dermal DCs and basophils that contribute to the generation of Th2 cells and T follicular helper (Tfh) cells that support IgE class-switching. Sensitization suppresses the generation of Tregs in the intestine in part through IL-4 production by mucosal mast cells. SEB, staphylococcal enterotoxin B.

[75, 76], but significance was lost after adjustment for household peanut consumption. In contrast, the significant association of household peanut consumption with peanut allergy remained after adjustment for maternal consumption. Household consumption of peanut was later reported to be correlated with peanut present in household dust, and the amount of peanut antigen found was biologically active as shown in BATs [77]. Several groups have now shown that food antigens can be readily detected in household dust [78, 79]. When studying T-cell responses to peanut in allergic individuals, the proliferative capacity was found to reside in the subset expressing the skin-homing molecule CLA [80], but not in the subset expressing the integrin $\beta 7$, a component of the intestinal homing molecule $\alpha 4\beta 7$. Homing capacity of lymphocytes is believed to reflect

the initial site of priming, and therefore, these data were used to argue for primary sensitization occurring through the skin. CCR4, another skin-homing molecule, has been identified as highly expressed on Ara h 1-specific T cells [24]. Thus, a growing body of work strongly suggests that the skin is a likely site of sensitization, at least to peanut.

Another important factor in sensitization beyond exposure is the immune milieu of the skin. As previously described, eczema is a risk factor for food allergy. Mutations in filaggrin, a protein that contributes to skin barrier function, have been shown to be associated with peanut allergy and sensitization [81, 82]. Recently, it was shown that the elevated risk of peanut allergy by exposure to peanut in household dust was present only in those with filaggrin mutations [83]. There are likely

many other factors that contribute to the barrier and immune milieu of the skin beyond filaggrin. Experimental models in mice support the concept that the skin functions as a primary site of sensitization to foods. Mice with filaggrin mutations have increased susceptibility to sensitization when allergen is applied topically [84]. Tape stripping, which models excoriations in atopic dermatitis and induces inflammation, also promotes sensitization to allergens applied topically [85, 86]. Peanut and tree nut allergens have intrinsic adjuvant activity in the skin that promote sensitization when applied topically without tape stripping or adjuvant, while other allergens such as soya or milk require adjuvant [87, 88]. This suggests that skin exposure as a risk factor for food allergy may be allergen-dependent. The epidemiologic data supporting a role for skin sensitization has primarily focused on peanut allergy.

Sensitization through the skin is dependent on epithelial-derived Th2-promoting cytokines. Thymic stromal lymphopoietin (TSLP) is central to sensitization induced by damage, either through tape stripping [86] or application of the Vitamin D3 analog calcipotriol [89]. TSLP is elevated in the skin of patients with atopic dermatitis [90]. In mice, skin TSLP drives sensitization by acting directly on skin DCs [91]. TSLP has also been shown to drive basophil accumulation into inflamed skin, and depletion of basophils suppresses sensitization to topical antigen [89, 91]. In the absence of overt damage, IL-33 and its receptor ST2 have been shown to play a critical role in sensitization to peanut through the skin, also via skin DCs [87]. In addition to adjuvant activity provided by damage or intrinsic to the allergens themselves, bacterial toxins including *Staphylococcus enterotoxin B* (SEB) can drive sensitization to bystander antigens by the induction of T follicular helper cells and Th2 cells in an IL-1-, IL-6-, and ST2-dependent pathway [87]. Clearly, multiple immune pathways relevant to eczema can drive sensitization to food allergens found in peanut and egg. Figure 1 summarizes the major pathways involved in site-specific induction of tolerance and sensitization to foods.

There are several implications of this model of immunopathophysiology of food allergy for prevention. The first is that exposure to allergens through the oral route, proposed to be inherently tolerizing, would prevent food allergy. This was first suggested by the observation that the rates of peanut allergy were very low in populations that practiced early introduction of peanut into the diet [92]. This year, the findings of the LEAP study were published and convincingly demonstrated in a cohort of 530 infants at increased risk for peanut allergy that early oral peanut introduction (at age 4–11 months) had a significant protective effect on the development of peanut allergy [93]. This clinical study

clearly shows that oral exposure to peanut is tolerizing in infants with elevated risk for peanut allergy. Very early exposure to cow's milk (in the first 2 weeks of life) was reported to be protective for milk allergy in a large (> 13 000 infants) population-based prospective study from Israel [94]. Early introduction of egg into the diet of infants with moderate-to-severe eczema at 4 months of age also showed a trend towards reduction of egg allergy although the trial was terminated early and was therefore underpowered [95]. At 4 months of age, many (36%) infants were found to be already sensitized to egg, and 31% of infants randomized to receive egg had an allergic reaction to egg powder. Sensitization occurring so early in life presents a challenge to oral tolerance as a prevention strategy. In addition to early oral introduction of allergens, interventions aimed at improving the barrier function of the skin may be effective for the prevention of allergy to peanuts or other foods. The use of emollients from birth onwards was recently shown to prevent the development of eczema in two studies [96, 97]. The proportion of infants with positive sensitization to egg (measured using an allergen microarray rather than standard ImmunoCAP approaches) was not significantly different between groups who did or did not receive skincare intervention, although the rates of sensitization were higher in those with eczema [97].

Sensitization to foods by the oral route

Although there is compelling evidence that sensitization to peanut may occur through skin exposure, there is also likely contribution of the mucosal immune system in food allergy. Cow's milk is most often the earliest food introduced into the diet, and milk allergy is the most common allergy of early childhood. Eigenmann et al. [98] demonstrated that T cells from milk-allergic donors stimulated with milk antigen *in vitro* upregulated the gut-homing marker $\alpha 4\beta 7$, which was not seen with the control antigen tetanus or in milk-tolerant controls. This is suggestive of gastrointestinal immune priming to milk. Therefore, it is likely that sensitization may occur in the gastrointestinal tract to some foods. Further evidence supporting an oral route of sensitization comes from the finding that antacid treatment of mice and humans increases the level of sensitization to food antigens [99, 100].

Mice are orally sensitized to foods by co-administration of adjuvants, commonly bacterial toxins such as CT or SEB. Administration of CT promotes IL-33 production from the intestinal epithelium and drives a migration of DCs from the lamina propria to the MLN [101]. Normally, tolerogenic CD103⁺ DCs increase their expression of OX40L, which is responsible for driving Th2 polarization [102]. Recently, it was shown that oral

administration of peanut and CT led to activation and degranulation of the resident intestinal population of eosinophils, which was responsible for driving DCs from the small intestine to the MLN [103]. Eosinophil peroxidase, a major degranulation product of eosinophils, altered the expression of co-stimulatory molecules and cytokines by mouse and human DCs. Uric acid, a metabolite produced upon cellular injury, is also elevated in mice fed with peanut and CT and contributes to sensitization through its adjuvant activity [104]. Therefore, tissue damage may also be a driving factor in sensitization through the gastrointestinal tract, similar to the skin.

In addition to activation of pathways that promote sensitization, adjuvants may work by suppressing regulatory pathways. Ganeshan et al. [71] found that sensitization of mice to ovalbumin using SEB was associated with a suppressed mRNA expression of TGF β in spleen cells. Administration of anti-CTLA-4 antibody has been used to suppress the function of Tregs during oral sensitization with CT. It was found that suppression of CTLA-4 enhanced sensitization when CT was present, but did not turn a tolerance response into sensitization [105]. In very interesting recently published findings, Noval Rivas et al. [106] elegantly demonstrate that sensitization via the oral route is a result of an interaction between IL-4 signalling and Tregs. Mice that carry a gain-of-function mutation in the IL-4 receptor, but not wild-type mice, develop sensitization to OVA when fed with OVA and SEB and develop anaphylaxis upon OVA challenge. These susceptible mice have a defect in Tregs in the intestine at baseline that is magnified after sensitization. Mast cells producing IL-4 suppress the generation of Tregs in the intestine [107]. Furthermore, Tregs in these susceptible mice deviate into IL-4-producing cells with reduced suppressive capacity. Transfer of Tregs from wild-type mice can restore tolerance in these mice. These data show that the balance between Th2 and Treg pathways is critical for maintenance of intestinal homeostasis, and also help to explain why tolerance is difficult to achieve once a Th2-biased immunity has already been established.

Role of microbiome

The increasing prevalence of food allergy documented in countries including the US and UK suggests an important contribution from environmental or lifestyle factors. The hygiene hypothesis, as originally proposed by Strachan to explain the increasing prevalence of allergic rhinitis [108], is likely also related to food allergy. The hygiene hypothesis has evolved over time, from focusing on infectious episodes in childhood to examining the sustained influence of commensal microbiota [109]. Having older siblings and living in a home

with pets are two factors associated with a lower risk of food allergy [110]. Pet ownership was recently elegantly tied to differences in the microbial composition of house dust [111], and transfer of microbiota or a single protective organism *Lactobacillus johnsonii* to mice was able to suppress experimental airway disease [112]. There are mixed reports about the impact of mode of birth (vaginal birth vs. Caesarean section) on susceptibility to food allergy [113, 114]. Mode of delivery clearly influences the microbial colonization of the infant, with Caesarean-born infants having an intestinal colonization reflecting the mother's skin microbiota [115, 116]. In addition to mode of delivery, early-life nutrition is another critical element determining the make-up of the infant intestinal microbiota [116].

The commensal microbiota provides a brake that suppresses IgE production. Germ-free mice have elevated levels of IgE, and reconstitution of germ-free mice with a normal microbiota in early life but not in adulthood suppresses IgE [117, 118]. Cahenzli et al. [118] demonstrated that diversity of colonization was necessary for suppression of IgE and prevention of food allergy in mice. Two recent human cohort studies have identified a reduced gastrointestinal microbial diversity in early life as being associated with the development of food allergy or food sensitization [119, 120]. It is not clear whether microbial diversity is in itself protective, perhaps by leading to increased diversity of the regulatory T-cell repertoire, or whether less diverse commensal populations lack critical protective species. There is evidence for some bacterial species having a protective effect in the context of food allergy. Atarashi et al. [121, 122] identified *Clostridium* species that could drive Treg expansion in the large intestine, and found that administration of either mouse or human *Clostridium* species could suppress the development of intestinal symptoms in a model of food allergy. Similarly, Stefká et al. [123] identified that the protective capacity of the microbiota in the prevention of peanut anaphylaxis in mice was conferred by *Clostridium* species. Microbial-induced protection from food allergy was associated with elevated Tregs, IgA, and epithelial barrier function. A number of different bacterial species have been shown to drive Treg expansion in the gastrointestinal tract [124], and this may be due to their ability to generate Treg-inducing short-chain fatty acids such as butyrate and propionate [125]. Data from human studies have shown an increased abundance of Clostridial cluster I, the core genus of the family Clostridiaceae [119] in children with food allergy. Although this appears to contradict the regulatory role of Clostridia species in food allergy, the immunoregulatory properties are likely strain specific.

In addition to microbes that may prevent food allergy, Noval Rivas et al. [126] demonstrated that

susceptibility to food allergy in mice was transmissible by the microbiota. As previously described, mice harbouring an activating mutation in the IL-4 receptor are susceptible to oral sensitization by feeding OVA and SEB. These susceptible mice have a different microbial signature upon sensitization, and transfer of this 'susceptible and sensitized' microbiota to germ-free mice renders the recipients susceptible to food allergy. It is clear that there is a dynamic crosstalk between the mucosal immune system and the microbiota. In designing human studies, it will be important to examine the microbiota in early life prior to the development of sensitization to provide greater evidence for causality of microbial changes leading to food allergy. Secondly, it will be important to address the role of the commensal microbiota at other sites, particularly the skin given its likely role as a site of sensitization to foods.

Role of nutrients and dietary factors

The mucosal immune system is shaped not only by the microbiota, but also by the exposure to dietary components. Epidemiologic studies have shown that an increased dietary diversity in early life is protective against the development of food allergy [127]. This, like microbial diversity, may be protective by increasing the diversity of the Treg repertoire in the gastrointestinal tract. Alternatively, there may be dietary components that confer increased or decreased risk for the development of food allergy. A third possibility is that a diverse diet drives a diverse intestinal microbiota [116, 128], and therefore indirectly promotes a regulatory tone in the intestine. There is relatively little information from either epidemiologic studies or mouse models to identify specific components of the diet that alter risk of food allergy development. Vitamin D deficiency was reported as associated with peanut sensitization (but not milk or egg sensitization) in a US cohort study of 3136 children and adolescents [129]. Stronger evidence for a role of Vitamin D in food allergy comes from Allen et al. [130], who found that low levels of Vitamin D (≤ 50 nmol/L) were associated with OFC-proven allergy to peanut and egg in an Australian cohort of 577 one-year-old infants. This finding was restricted to the subset with Australian-born parents ($n = 271$) and was not observed in the subset of infants with foreign-born parents. The vitamin D hypothesis is also supported by reports of association between food allergy or epinephrine prescriptions with season of birth [131] and geographical latitude [132, 133] (factors involved in exposure to UV light and regulation of vitamin D levels). Fall birth is associated with increased risk of food allergy. Although several factors may contribute to this increased risk, including viral infection as a contributor to priming, the increased risk was only

observed among Caucasians, in whom vitamin D levels are more readily regulated by UV exposure [131]. In addition, impact of season of birth was limited to those with a history of eczema, implicating an interaction between UV exposure and the immune or barrier function of the skin. UV radiation promotes the generation of antigen-specific regulatory T cells and may prevent the development of sensitization via the skin [134]. In addition to vitamin D deficiency, high folate levels in early life have been described to be associated with higher rates of food sensitization [135]. High-fat diet influences both the mucosal immune system and the composition of the microbiota [136], and obesity has been shown to be a risk factor for food sensitization [137]. Experimental models of food allergy or oral tolerance in mice have identified protective roles for vitamin A [138], polyunsaturated fatty acids [139], and aryl hydrocarbon receptor ligands [140], the latter being produced by cruciferous vegetables such as broccoli, cauliflower, and cabbage.

The first dietary intake of the infant is most commonly breastmilk. Breastmilk contains antibodies, cytokines, oligosaccharides, anti-microbial proteins, and fatty acids. Infants receiving breastmilk vs. formula have significant differences in their gastrointestinal microbial composition and immune response [141]. There is a lack of conclusive data on the protective role of breastfeeding in food allergy in human cohorts [142]. In the context of food allergy, mothers may restrict breastfeeding when infants show early symptoms, which can make results more difficult to interpret. The lack of conclusive findings may also be due to differences in the composition of breastmilk that are not reflected in studies that look merely at the presence or absence of breastfeeding. Lower levels of cow's milk-specific IgA as well as low cytokine levels that may contribute to the maturation of the infant mucosal immune system have been identified in breastmilk of mothers of infants who develop cow's milk allergy, compared to mothers of non-milk-allergic controls [143, 144].

Genetics of food allergy

In addition to environmental risk factors, there is a strong genetic contribution to susceptibility to food allergy. Sicherer et al. [145] found a concordance rate of peanut allergy of 64.3% in monozygotic twins compared to 6.8% in dizygotic twins. Using a candidate gene approach, Howell et al. [146] found significant differences between HLA Class II DRB1, DQB1, and DPB1 genotype frequencies in peanut-allergic cases vs. controls in a UK-based study. Madore et al. [147] also identified association of HLA genes with peanut allergy in a case-control Canadian study. A recent GWAS study utilized a US-based birth cohort of 1315 children and 1444 parents to identify variants in HLA-DQ and

HLA-DR that conferred increased risk of peanut allergy, but not milk or egg allergy [148]. Variants were associated with modifications in methylation status of the genes. In contrast to the results above, two studies using siblings [149] or twins [150] discordant for peanut allergy did not identify HLA variants associated with peanut allergy, although HLA DRB1*0803 was more frequent in families with peanut allergy compared to a reference population [149]. Variants in HLA genes may alter the epitopes of peanut that are presented to T cells and may select for epitopes that are better able to induce Th2-skewed responses, although clearly additional susceptibility factors appear to be required.

In addition to HLA genes, filaggrin mutations are associated with increased risk of peanut allergy or peanut sensitization [81]. Filaggrin is a protein that contributes to the barrier function of the skin, as outlined previously. Filaggrin mutations also contribute to the risk of eczema, but independently increase the risk of peanut allergy. In addition to skin expression, filaggrin is expressed in the oral mucosa, and there are mixed reports of filaggrin expression in the oesophagus, such that filaggrin mutations could potentially contribute to sensitization upon oral exposure [151, 152].

Natural resolution of food allergy

Different food allergies have different rates of resolution, with milk and egg allergy being commonly outgrown [153, 154] while peanut and tree nut allergy are outgrown in a minority (10–20%) of cases [155]. Most milk- and egg-allergic children will tolerate extensively heated forms of milk [156] and egg [157], respectively, and these children resolve their allergy more rapidly than those who react to extensively heated forms of milk or egg [158, 159]. Baked-milk-tolerant children have a greater frequency of milk-specific Tregs [28], suggesting an active process of immune tolerance. Similarly, individuals who have outgrown a non-IgE-mediated milk allergy have an increased frequency of milk-specific Tregs after an oral milk challenge [29]. When following the natural history of a cohort of egg-allergic children, 49% had outgrown their egg allergy at a median age of 72 months [153]. Factors predictive of outgrowth included low baseline egg-specific IgE levels and reac-

tions limited to acute skin symptoms. Low levels of egg-induced IL-4 gene expression in T cells were also predictive of outgrowth. Similar findings were reported for milk [154], with 53% outgrowing milk allergy in a median of 66 months. Levels of milk-specific IgE had the greatest predictive value for allergy resolution. The biology of long-lived IgE-producing plasma cells is very poorly understood, but is likely a key factor in this phenomenon of resolution. The role of Th2 cells, Tregs, or antigen exposure in the maintenance or loss of long-lived IgE-producing plasma cells is not known. Understanding the immune mechanisms of natural resolution of food allergy may provide the best information about immune mechanisms that lead to lasting tolerance to foods, leading to therapeutic approaches more successful in the establishment of true tolerance than allergen immunotherapy approaches currently under investigation [160, 161].

Conclusions

We are beginning to develop a more comprehensive understanding of factors that contribute to the development of sensitization and tolerance to foods. Recent work has changed the paradigm of peanut allergy, providing evidence that the skin is an important site of primary sensitization to peanut and that early oral introduction of peanut into the diet can prevent peanut allergy (see Fig. 1). In this schematic, the induction of immune responses to peanut via mildly inflamed skin, resulting in IgE production and generation of Th2/Th9/Tfh cells, will subsequently alter the immune milieu of the gastrointestinal tract such that oral exposure is no longer tolerizing. Future work will need to address how this paradigm fits with other common food allergies. Successful treatment of established food allergy remains a challenge. Longitudinal immune profiling of individuals who achieve lasting tolerance naturally is needed in order to understand mechanisms of food allergy resolution and to better design successful strategies for the treatment of food allergy.

Conflict of interest

The author has no financial conflicts of interest.

References

- 1 Boyce JA, Assa'ad A, Burks AW *et al.* Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. *J Allergy Clin Immunol* 2010; 126:1105–18.
- 2 Berin MC. Immunopathophysiology of food protein-induced enterocolitis syndrome. *J Allergy Clin Immunol* 2015; 135:1108–13.
- 3 Rothenberg ME. Molecular, genetic, and cellular bases for treating eosinophilic esophagitis. *Gastroenterology* 2015; 148:1143–57.
- 4 Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; 107:891–6.
- 5 Peters RL, Allen KJ, Dharmage SC *et al.* Skin prick test responses and allergen-specific IgE levels as predictors of peanut, egg, and sesame

- allergy in infants. *J Allergy Clin Immunol* 2013; 132:874–80.
- 6 Celik-Bilgili S, Mehl A, Verstege A *et al.* The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. *Clin Exp Allergy* 2005; 35:268–73.
- 7 Komata T, Soderstrom L, Borres MP, Tachimoto H, Ebisawa M. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. *J Allergy Clin Immunol* 2007; 119:1272–4.
- 8 Keet CA, Wood RA, Matsui EC. Limitations of reliance on specific IgE for epidemiologic surveillance of food allergy. *J Allergy Clin Immunol* 2012; 130:1207–9.e10.
- 9 Lieberman JA, Glaumann S, Batelson S, Borres MP, Sampson HA, Nilsson C. The utility of peanut components in the diagnosis of IgE-mediated peanut allergy among distinct populations. *J Allergy Clin Immunol Pract* 2013; 1:75–82.
- 10 Asaranoj A, Nilsson C, Lidholm J *et al.* Peanut component Ara h 8 sensitization and tolerance to peanut. *J Allergy Clin Immunol* 2012; 130:468–72.
- 11 Shreffler WG, Beyer K, Chu TH, Burks AW, Sampson HA. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunol* 2004; 113:776–82.
- 12 Wang J, Lin J, Bardina L *et al.* Correlation of IgE/IgG4 milk epitopes and affinity of milk-specific IgE antibodies with different phenotypes of clinical milk allergy. *J Allergy Clin Immunol* 2010; 125:695–702, 702.e1–702.e6.
- 13 Shade KT, Platzter B, Washburn N *et al.* A single glycan on IgE is indispensable for initiation of anaphylaxis. *J Exp Med* 2015; 212:457–67.
- 14 Robertson MW, Liu FT. Heterogeneous IgE glycoforms characterized by differential recognition of an endogenous lectin (IgE-binding protein). *J Immunol* 1991; 147:3024–30.
- 15 Ford LS, Bloom KA, Nowak-Wegrzyn AH, Shreffler WG, Masilamani M, Sampson HA. Basophil reactivity, wheal size, and immunoglobulin levels distinguish degrees of cow's milk tolerance. *J Allergy Clin Immunol* 2012; 131:180–6.e1–3.
- 16 Santos AF, Douiri A, Becares N *et al.* Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J Allergy Clin Immunol* 2014; 134:645–52.
- 17 Santos AF, Du Toit G, Douiri A *et al.* Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. *J Allergy Clin Immunol* 2015; 135:179–86.
- 18 Blumchen K, Beder A, Beschoner J *et al.* Modified oral food challenge used with sensitization biomarkers provides more real-life clinical thresholds for peanut allergy. *J Allergy Clin Immunol* 2014; 134:390–8.
- 19 Burks AW, Jones SM, Wood RA *et al.* Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med* 2012; 367:233–43.
- 20 Kulis M, Saba K, Kim EH *et al.* Increased peanut-specific IgA levels in saliva correlate with food challenge outcomes after peanut sublingual immunotherapy. *J Allergy Clin Immunol* 2012; 129:1159–62.
- 21 Caubet JC, Bencharitiwong R, Moshier E, Godbold JH, Sampson HA, Nowak-Wegrzyn A. Significance of ovomucoid- and ovalbumin-specific IgE/IgG (4) ratios in egg allergy. *J Allergy Clin Immunol* 2012; 129:739–47.
- 22 Vazquez-Ortiz M, Pascal M, Jimenez-Feijoo R *et al.* Ovalbumin-specific IgE/IgG4 ratio might improve the prediction of cooked and uncooked egg tolerance development in egg-allergic children. *Clin Exp Allergy* 2014; 44:579–88.
- 23 Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. *J Clin Invest* 2003; 111:1065–72.
- 24 DeLong JH, Simpson KH, Wambre E, James EA, Robinson D, Kwok WW. Ara h 1-reactive T cells in individuals with peanut allergy. *J Allergy Clin Immunol* 2011; 127:1211–8.e3.
- 25 Prussin C, Lee J, Foster B. Eosinophilic gastrointestinal disease and peanut allergy are alternatively associated with IL-5+ and IL-5(–) T(H)2 responses. *J Allergy Clin Immunol* 2009; 124:1326–32.e6.
- 26 Sicherer SH, Wood RA, Stablein D *et al.* Immunologic features of infants with milk or egg allergy enrolled in an observational study (Consortium of Food Allergy Research) of food allergy. *J Allergy Clin Immunol* 2010; 125:1077–83.
- 27 Brough HA, Cousins DJ, Munteanu A *et al.* IL-9 is a key component of memory TH cell peanut-specific responses from children with peanut allergy. *J Allergy Clin Immunol* 2014; 134:1329–38.e10.
- 28 Shreffler WG, Wanich N, Moloney M, Nowak-Wegrzyn A, Sampson HA. Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. *J Allergy Clin Immunol* 2009; 123:43–52.e7.
- 29 Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004; 199:1679–88.
- 30 Shreffler WG, Castro RR, Kucuk ZY *et al.* The major glycoprotein allergen from *Arachis hypogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. *J Immunol* 2006; 177:3677–85.
- 31 Hsu SC, Chen CH, Tsai SH *et al.* Functional interaction of common allergens and a C-type lectin receptor, dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN), on human dendritic cells. *J Biol Chem* 2010; 285:7903–10.
- 32 Kean DE, Goodridge HS, McGuinness S, Harnett MM, Alcocer MJ, Harnett W. Differential polarization of immune responses by plant 2S seed albumins, Ber e 1, and SFA8. *J Immunol* 2006; 177:1561–6.
- 33 Maurer D, Fiebiger E, Reininger B *et al.* Fc epsilon receptor I on dendritic cells delivers IgE-bound multivalent antigens into a cathepsin S-dependent pathway of MHC class II presentation. *J Immunol* 1998; 161:2731–9.
- 34 Sallmann E, Reininger B, Brandt S *et al.* High-affinity IgE receptors on dendritic cells exacerbate Th2-dependent inflammation. *J Immunol* 2011; 187:164–71.
- 35 Schroeder JT, Bieneman AP, Chichester KL *et al.* Decreases in human dendritic cell-dependent T(H)2-like responses after acute in vivo IgE neutralization. *J Allergy Clin Immunol* 2010; 125:896–901.e6.

- 36 Gorelik M, Narisety SD, Guerrero AL *et al.* Suppression of the immunologic response to peanut during immunotherapy is often transient. *J Allergy Clin Immunol* 2014; 135: 1283–92.
- 37 Syed A, Garcia MA, Lyu SC *et al.* Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol* 2014; 133:500–10.e11.
- 38 Sun J, Arias K, Alvarez D *et al.* Impact of CD40 ligand, B cells, and mast cells in peanut-induced anaphylactic responses. *J Immunol* 2007; 179:6696–703.
- 39 Reber LL, Marichal T, Mukai K *et al.* Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice. *J Allergy Clin Immunol* 2013; 132:881–8.e1–11.
- 40 Arias K, Chu DK, Flader K *et al.* Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice. *J Allergy Clin Immunol* 2011; 127:1552–61.e1.
- 41 Savage JH, Courneya JP, Sterba PM, Macglashan DW, Saini SS, Wood RA. Kinetics of mast cell, basophil, and oral food challenge responses in omalizumab-treated adults with peanut allergy. *J Allergy Clin Immunol* 2012; 130:1123–9.e2.
- 42 Arias K, Baig M, Colangelo M *et al.* Concurrent blockade of platelet-activating factor and histamine prevents life-threatening peanut-induced anaphylactic reactions. *J Allergy Clin Immunol* 2009; 124:307–14, 314.e1–2.
- 43 Vadas P, Perelman B, Liss G. Platelet-activating factor, histamine, and trypsin levels in human anaphylaxis. *J Allergy Clin Immunol* 2012; 131:144–9.
- 44 Vadas P, Gold M, Perelman B *et al.* Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med* 2008; 358:28–35.
- 45 Finkelman FD. Anaphylaxis: lessons from mouse models. *J Allergy Clin Immunol* 2007; 120:506–15; quiz 16–7.
- 46 Osterfeld H, Ahrens R, Strait R, Finkelman FD, Renaud JC, Hogan SP. Differential roles for the IL-9/IL-9 receptor alpha-chain pathway in systemic and oral antigen-induced anaphylaxis. *J Allergy Clin Immunol* 2010; 125:469–76.e2.
- 47 Khodoun MV, Strait R, Armstrong L, Yanase N, Finkelman FD. Identification of markers that distinguish IgE- from IgG-mediated anaphylaxis. *Proc Natl Acad Sci U S A* 2011; 108: 12413–8.
- 48 Ahrens B, Niggemann B, Wahn U, Beyer K. Organ-specific symptoms during oral food challenge in children with food allergy. *J Allergy Clin Immunol* 2012; 130:549–51.
- 49 Roth-Walter F, Berin MC, Arnaboldi P *et al.* Pasteurization of milk proteins promotes allergic sensitization by enhancing uptake through Peyer's patches. *Allergy* 2008; 63:882–90.
- 50 Martos G, Lopez-Exposito I, Benchari-tiwong R, Berin MC, Nowak-Wegrzyn A. Mechanisms underlying differential food allergy response to heated egg. *J Allergy Clin Immunol* 2011; 127:990–7.e1–2.
- 51 Li J, Wang Y, Tang L *et al.* Dietary medium-chain triglycerides promote oral allergic sensitization and orally induced anaphylaxis to peanut protein in mice. *J Allergy Clin Immunol* 2013; 131:442–50.
- 52 Wells HG, Osborne TB. The biological reactions of the vegetable proteins. I. Anaphylaxis. *J Infect Dis* 1911; 8:66–124.
- 53 Kraus TA, Toy L, Chan L, Childs J, Mayer L. Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease. *Gastroenterology* 2004; 126: 1771–8.
- 54 Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto de Lafaille MA. Oral tolerance in the absence of naturally occurring Tregs. *J Clin Invest* 2005; 115:1923–33.
- 55 Hadis U, Wahl B, Schulz O *et al.* Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity* 2011; 34:237–46.
- 56 Weiner HL, da Cunha AP, Quintana F, Wu H. Oral tolerance. *Immunol Rev* 2011; 241:241–59.
- 57 Groux H, O'Garra A, Bigler M *et al.* A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997; 389:737–42.
- 58 Pabst O, Mowat AM. Oral tolerance to food protein. *Mucosal Immunol* 2012; 5:232–9.
- 59 Worbs T, Bode U, Yan S *et al.* Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* 2006; 203:519–27.
- 60 Johansson-Lindbom B, Svensson M, Pabst O *et al.* Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med* 2005; 202:1063–73.
- 61 Jaensson E, Uronen-Hansson H, Pabst O *et al.* Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. *J Exp Med* 2008; 205:2139–49.
- 62 Coombes JL, Siddiqui KR, Arancibia-Carcamo CV *et al.* A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007; 204:1757–64.
- 63 Matteoli G, Mazzini E, Iliev ID *et al.* Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut* 2010; 59:595–604.
- 64 Bogunovic M, Ginhoux F, Helft J *et al.* Origin of the lamina propria dendritic cell network. *Immunity* 2009; 31:513–25.
- 65 Miller JC, Brown BD, Shay T *et al.* Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol* 2012; 13:888–99.
- 66 Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1 (+) macrophages to CD103(+) dendritic cells. *Immunity* 2014; 40:248–61.
- 67 McDole JR, Wheeler LW, McDonald KG *et al.* Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* 2012; 483:345–9.
- 68 Shan M, Gentile M, Yeiser JR *et al.* Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. *Science* 2013; 342:447–53.
- 69 Mortha A, Chudnovskiy A, Hashimoto D *et al.* Microbiota-dependent cross-talk between macrophages and ILC3 promotes intestinal homeostasis. *Science* 2014; 343:1249288.

- 70 Berin MC, Wang W. Reduced severity of peanut-induced anaphylaxis in TLR9-deficient mice is associated with selective defects in humoral immunity. *Mucosal Immunol* 2013; 6:114–21.
- 71 Ganesan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ. Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J Allergy Clin Immunol* 2009; 123:231–8.e4.
- 72 Martin PE, Eckert JK, Koplin JJ *et al.* Which infants with eczema are at risk of food allergy? Results from a population-based cohort. *Clin Exp Allergy* 2015; 45:255–64.
- 73 Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy in childhood. *N Engl J Med* 2003; 348:977–85.
- 74 Fukutomi Y, Taniguchi M, Nakamura H, Akiyama K. Epidemiological link between wheat allergy and exposure to hydrolyzed wheat protein in facial soap. *Allergy* 2014; 69:1405–11.
- 75 Fox AT, Sasieni P, du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. *J Allergy Clin Immunol* 2009; 123:417–23.
- 76 Sicherer SH, Wood RA, Stablein D *et al.* Maternal consumption of peanut during pregnancy is associated with peanut sensitization in atopic infants. *J Allergy Clin Immunol* 2010; 126:1191–7.
- 77 Brough HA, Santos AF, Makinson K *et al.* Peanut protein in household dust is related to household peanut consumption and is biologically active. *J Allergy Clin Immunol* 2013; 132:630–8.
- 78 Trendelenburg V, Ahrens B, Wehrmann AK, Kalb B, Niggemann B, Beyer K. Peanut allergen in house dust of eating area and bed – a risk factor for peanut sensitization? *Allergy* 2013; 68:1460–2.
- 79 Bertelsen RJ, Faeste CK, Granum B *et al.* Food allergens in mattress dust in Norwegian homes – a potentially important source of allergen exposure. *Clin Exp Allergy* 2014; 44:142–9.
- 80 Chan SM, Turcanu V, Stephens AC, Fox AT, Grieve AP, Lack G. Cutaneous lymphocyte antigen and alpha4beta7 T-lymphocyte responses are associated with peanut allergy and tolerance in children. *Allergy* 2012; 67:336–42.
- 81 Brown SJ, Asai Y, Cordell HJ *et al.* Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol* 2011; 127:661–7.
- 82 Tan HT, Ellis JA, Koplin JJ *et al.* Filaggrin loss-of-function mutations do not predict food allergy over and above the risk of food sensitization among infants. *J Allergy Clin Immunol* 2012; 130:1211–3.e3.
- 83 Brough HA, Simpson A, Makinson K *et al.* Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. *J Allergy Clin Immunol* 2014; 134:867–75.e1.
- 84 Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. *J Allergy Clin Immunol* 2009; 124:485–93, 493.e1.
- 85 He R, Oyoshi MK, Garibyan L, Kumar L, Ziegler SF, Geha RS. TSLP acts on infiltrating effector T cells to drive allergic skin inflammation. *Proc Natl Acad Sci U S A* 2008; 105:11875–80.
- 86 Oyoshi MK, Larson RP, Ziegler SF, Geha RS. Mechanical injury polarizes skin dendritic cells to elicit a T(H)2 response by inducing cutaneous thymic stromal lymphopoietin expression. *J Allergy Clin Immunol* 2010; 126:976–84, 984.e1–5.
- 87 Tordesillas L, Goswami R, Benede S *et al.* Skin exposure promotes a Th2-dependent sensitization to peanut allergens. *J Clin Invest* 2014; 124:4965–75.
- 88 Dunkin D, Berin MC, Mayer L. Allergic sensitization can be induced via multiple physiologic routes in an adjuvant-dependent manner. *J Allergy Clin Immunol* 2011; 128:1251–8.e2.
- 89 Noti M, Kim BS, Siracusa MC *et al.* Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J Allergy Clin Immunol* 2014; 133:1390–9, 1399.e1–6.
- 90 Soumelis V, Reche PA, Kanzler H *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; 3:673–80.
- 91 Han H, Thelen TD, Comeau MR, Ziegler SF. Thymic stromal lymphopoietin-mediated epicutaneous inflammation promotes acute diarrhea and anaphylaxis. *J Clin Invest* 2014; 124:5442–52.
- 92 Du Toit G, Katz Y, Sasieni P *et al.* Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol* 2008; 122:984–91.
- 93 Du Toit G, Roberts G, Sayre PH *et al.* Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015; 372:803–13.
- 94 Katz Y, Rajuan N, Goldberg MR *et al.* Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. *J Allergy Clin Immunol* 2010; 126:77–82.e1.
- 95 Palmer DJ, Metcalfe J, Makrides M *et al.* Early regular egg exposure in infants with eczema: a randomized controlled trial. *J Allergy Clin Immunol* 2013; 132:387–92.e1.
- 96 Simpson EL, Chalmers JR, Hanifin JM *et al.* Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J Allergy Clin Immunol* 2014; 134:818–23.
- 97 Horimukai K, Morita K, Narita M *et al.* Application of moisturizer to neonates prevents development of atopic dermatitis. *J Allergy Clin Immunol* 2014; 134:824–30.e6.
- 98 Eigenmann PA, Tropia L, Hauser C. The mucosal adhesion receptor alpha4beta7 integrin is selectively increased in lymphocytes stimulated with beta-lactoglobulin in children allergic to cow's milk. *J Allergy Clin Immunol* 1999; 103:931–6.
- 99 Untersmayr E, Scholl I, Swoboda I *et al.* Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. *J Allergy Clin Immunol* 2003; 112:616–23.
- 100 Scholl I, Untersmayr E, Bakos N *et al.* Antiulcer drugs promote oral sensitization and hypersensitivity to hazelnut allergens in BALB/c mice and humans. *Am J Clin Nutr* 2005; 81:154–60.
- 101 Chu DK, Llop-Guevara A, Walker TD *et al.* IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization.

- J Allergy Clin Immunol* 2012; 131:187–200.e1–8.
- 102 Blazquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol* 2008; 180:4441–50.
 - 103 Chu DK, Jimenez-Saiz R, Verschoor CP *et al.* Indigenous enteric eosinophils control DCs to initiate a primary Th2 immune response in vivo. *J Exp Med* 2014; 211:1657–72.
 - 104 Kong J, Chalcraft K, Mandur TS *et al.* Comprehensive metabolomics identifies the alarmin uric acid as a critical signal for the induction of peanut allergy. *Allergy* 2015; 70:495–505.
 - 105 van Wijk F, Hoeks S, Nierkens S *et al.* CTLA-4 signaling regulates the intensity of hypersensitivity responses to food antigens, but is not decisive in the induction of sensitization. *J Immunol* 2005; 174:174–9.
 - 106 Noval Rivas M, Burton OT, Wise P *et al.* Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* 2015; 42:512–23.
 - 107 Burton OT, Noval Rivas M, Zhou JS *et al.* Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity* 2014; 41:141–51.
 - 108 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299:1259–60.
 - 109 West CE, Renz H, Jenmalm MC *et al.* The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. *J Allergy Clin Immunol* 2015; 135:3–13; quiz 14.
 - 110 Peters RL, Allen KJ, Dharmage SC *et al.* Differential factors associated with challenge-proven food allergy phenotypes in a population cohort of infants: a latent class analysis. *Clin Exp Allergy* 2014; 45:953–63.
 - 111 Fujimura KE, Johnson CC, Ownby DR *et al.* Man's best friend? The effect of pet ownership on house dust microbial communities. *J Allergy Clin Immunol* 2010; 126:410–2, 412.e1–3.
 - 112 Fujimura KE, Demoor T, Rauch M *et al.* House dust exposure mediates gut microbiome *Lactobacillus* enrichment and airway immune defense against allergens and virus infection. *Proc Natl Acad Sci U S A* 2014; 111:805–10.
 - 113 Eggesbo M, Botten G, Stigum H, Nafstad P, Magnus P. Is delivery by cesarean section a risk factor for food allergy? *J Allergy Clin Immunol* 2003; 112:420–6.
 - 114 Koplin JJ, Dharmage SC, Ponsonby AL *et al.* Environmental and demographic risk factors for egg allergy in a population-based study of infants. *Allergy* 2012; 67:1415–22.
 - 115 Dominguez-Bello MG, Costello EK, Contreras M *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010; 107:11971–5.
 - 116 Backhed F, Roswall J, Peng Y *et al.* Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* 2015; 17:690–703.
 - 117 Hill DA, Siracusa MC, Abt MC *et al.* Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med* 2012; 18:538–46.
 - 118 Cahenzli J, Koller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe* 2013; 14:559–70.
 - 119 Ling Z, Li Z, Liu X *et al.* Altered fecal microbiota composition associated with food allergy in infants. *Appl Environ Microbiol* 2014; 80:2546–54.
 - 120 Azad MB, Konya T, Guttman DS *et al.* Infant gut microbiota and food sensitization: associations in the first year of life. *Clin Exp Allergy* 2015; 45:632–43.
 - 121 Atarashi K, Tanoue T, Shima T *et al.* Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* 2011; 331:337–41.
 - 122 Atarashi K, Tanoue T, Oshima K *et al.* Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* 2013; 500:232–6.
 - 123 Stefka AT, Feehley T, Tripathi P *et al.* Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U S A* 2014; 111:13145–50.
 - 124 Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JI. Identifying gut microbe–host phenotype relationships using combinatorial communities in gnotobiotic mice. *Sci Transl Med* 2014; 6:220ra11.
 - 125 Smith PM, Howitt MR, Panikov N *et al.* The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 2013; 341:569–73.
 - 126 Noval Rivas M, Burton OT, Wise P *et al.* A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol* 2013; 131:201–12.
 - 127 Roduit C, Frei R, Depner M *et al.* Increased food diversity in the first year of life is inversely associated with allergic diseases. *J Allergy Clin Immunol* 2014; 133:1056–64.
 - 128 Koenig JE, Spor A, Scalfone N *et al.* Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A* 2011; 108(Suppl. 1):4578–85.
 - 129 Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML. Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005–2006. *J Allergy Clin Immunol* 2011; 127:1195–202.
 - 130 Allen KJ, Koplin JJ, Ponsonby AL *et al.* Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J Allergy Clin Immunol* 2013; 131:1109–16, 1116.e1–6.
 - 131 Keet CA, Matsui EC, Savage JH *et al.* Potential mechanisms for the association between fall birth and food allergy. *Allergy* 2012; 67:775–82.
 - 132 Camargo CA Jr, Clark S, Kaplan MS, Lieberman P, Wood RA. Regional differences in EpiPen prescriptions in the United States: the potential role of vitamin D. *J Allergy Clin Immunol* 2007; 120:131–6.
 - 133 Mullins RJ, Clark S, Camargo CA Jr. Regional variation in epinephrine autoinjector prescriptions in Australia: more evidence for the vitamin D-anaphylaxis hypothesis. *Ann Allergy Asthma Immunol* 2009; 103:488–95.
 - 134 Schwarz A, Maeda A, Schwarz T. Alteration of the migratory behavior of UV-induced regulatory T cells by tissue-specific dendritic cells. *J Immunol* 2007; 178:877–86.
 - 135 Okupa AY, Lemanske RF Jr, Jackson DJ, Evans MD, Wood RA, Matsui EC.

- Early-life folate levels are associated with incident allergic sensitization. *J Allergy Clin Immunol* 2013; 131:226–8.e1–2.
- 136 Upadhyay V, Poroyko V, Kim TJ *et al.* Lymphotoxin regulates commensal responses to enable diet-induced obesity. *Nat Immunol* 2012; 13:947–53.
 - 137 Visness CM, London SJ, Daniels JL *et al.* Association of obesity with IgE levels and allergy symptoms in children and adolescents: results from the National Health and Nutrition Examination Survey 2005–2006. *J Allergy Clin Immunol* 2009; 123:1163–9.
 - 138 Cassani B, Villablanca EJ, Quintana FJ *et al.* Gut-tropic T cells that express integrin alpha4beta7 and CCR9 are required for induction of oral immune tolerance in mice. *Gastroenterology* 2011; 141:2109–18.
 - 139 van den Elsen LW, van Esch BC, Hofman GA *et al.* Dietary long chain n-3 polyunsaturated fatty acids prevent allergic sensitization to cow's milk protein in mice. *Clin Exp Allergy* 2013; 43:798–810.
 - 140 Schulz VJ, Smit JJ, Willemsen KJ *et al.* Activation of the aryl hydrocarbon receptor suppresses sensitization in a mouse peanut allergy model. *Toxicol Sci* 2011; 123:491–500.
 - 141 Schwartz S, Friedberg I, Ivanov IV *et al.* A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. *Genome Biol* 2012; 13:r32.
 - 142 Matheson MC, Allen KJ, Tang ML. Understanding the evidence for and against the role of breastfeeding in allergy prevention. *Clin Exp Allergy* 2012; 42:827–51.
 - 143 Jarvinen KM, Westfall JE, Seppo MS *et al.* Role of maternal elimination diets and human milk IgA in the development of cow's milk allergy in the infants. *Clin Exp Allergy* 2014; 44:69–78.
 - 144 Jarvinen KM, Suarez-Farinas M, Savilahti E, Sampson HA, Berin MC. Immune factors in breast milk related to infant milk allergy are independent of maternal atopy. *J Allergy Clin Immunol* 2014; 135:1390–3.e1–6.
 - 145 Sicherer SH, Furlong TJ, Maes HH, Desnick RJ, Sampson HA, Gelb BD. Genetics of peanut allergy: a twin study. *J Allergy Clin Immunol* 2000; 106:53–6.
 - 146 Howell WM, Turner SJ, Hourihane JO, Dean TP, Warner JO. HLA class II DRB1, DQB1 and DPB1 genotypic associations with peanut allergy: evidence from a family-based and case-control study. *Clin Exp Allergy* 1998; 28:156–62.
 - 147 Madore AM, Vaillancourt VT, Asai Y *et al.* HLA-DQB1*02 and DQB1*06:03P are associated with peanut allergy. *Eur J Hum Genet* 2013; 21:1181–4.
 - 148 Hong X, Hao K, Ladd-Acosta C *et al.* Genome-wide association study identifies peanut allergy-specific loci and evidence of epigenetic mediation in US children. *Nat Commun* 2015; 6:6304.
 - 149 Dreskin SC, Tripputi MT, Aubrey MT *et al.* Peanut-allergic subjects and their peanut-tolerant siblings have large differences in peanut-specific IgG that are independent of HLA class II. *Clin Immunol* 2010; 137:366–73.
 - 150 Shreffler WG, Charlop-Powers Z, Sicherer SH. Lack of association of HLA class II alleles with peanut allergy. *Ann Allergy Asthma Immunol* 2006; 96:865–9.
 - 151 De Benedetto A, Qualia CM, Baroody FM, Beck LA. Filaggrin expression in oral, nasal, and esophageal mucosa. *J Invest Dermatol* 2008; 128:1594–7.
 - 152 Blanchard C, Stucke EM, Burwinkel K *et al.* Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. *J Immunol* 2010; 184:4033–41.
 - 153 Sicherer SH, Wood RA, Vickery BP *et al.* The natural history of egg allergy in an observational cohort. *J Allergy Clin Immunol* 2014; 133:492–9.e8.
 - 154 Wood RA, Sicherer SH, Vickery BP *et al.* The natural history of milk allergy in an observational cohort. *J Allergy Clin Immunol* 2013; 131:805–12.
 - 155 Peters RL, Allen KJ, Dharmage SC *et al.* Natural history of peanut allergy and predictors of resolution in the first 4 years of life: a population-based assessment. *J Allergy Clin Immunol* 2015; 135:1257–66.e1–2.
 - 156 Nowak-Węgrzyn A, Bloom KA, Sicherer SH *et al.* Tolerance to extensively heated milk in children with cow's milk allergy. *J Allergy Clin Immunol* 2008; 122:342–7, 347.e1–2.
 - 157 Lemon-Mule H, Sampson HA, Sicherer SH, Shreffler WG, Noone S, Nowak-Węgrzyn A. Immunologic changes in children with egg allergy ingesting extensively heated egg. *J Allergy Clin Immunol* 2008; 122:977–83.e1.
 - 158 Kim JS, Nowak-Węgrzyn A, Sicherer SH, Noone S, Moshier EL, Sampson HA. Dietary baked milk accelerates the resolution of cow's milk allergy in children. *J Allergy Clin Immunol* 2011; 128:125–31.e2.
 - 159 Leonard SA, Sampson HA, Sicherer SH *et al.* Dietary baked egg accelerates resolution of egg allergy in children. *J Allergy Clin Immunol* 2012; 130:473–80.e1.
 - 160 Nowak-Węgrzyn A, Albin S. Oral immunotherapy for food allergy: mechanisms and role in management. *Clin Exp Allergy* 2015; 45:368–83.
 - 161 Keet CA, Wood RA. Emerging therapies for food allergy. *J Clin Invest* 2014; 124:1880–6.