Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2022 23(9):705-731
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2200047

Biomarkers in oral immunotherapy

Haitao ZHU[1], Kaifa TANG[2], Guoqiang CHEN[1], Zhongwei LIU[3]✉

[1]*Department of Pediatrics (No. 3 Ward), Northwest Women's and Children's Hospital, Xi'an 710061, China*
[2]*Department of Urology, the Affiliated Hospital of Guizhou Medical University, Guiyang 550004, China*
[3]*Department of Cardiology, Shaanxi Provincial People's Hospital, Xi'an 710068, China*

Abstract: Food allergy (FA) is a global health problem that affects a large population, and thus effective treatment is highly desirable. Oral immunotherapy (OIT) has been showing reasonable efficacy and favorable safety in most FA subjects. Dependable biomarkers are needed for treatment assessment and outcome prediction during OIT. Several immunological indicators have been used as biomarkers in OIT, such as skin prick tests, basophil and mast cell reactivity, T cell and B cell responses, allergen-specific antibody levels, and cytokines. Other novel indicators also could be potential biomarkers. In this review, we discuss and assess the application of various immunological indicators as biomarkers for OIT.

Key words: Food allergy; Oral immunotherapy; Biomarker; Immune tolerance; Desensitization

1 Introduction

Food allergy (FA) has become a major public health problem, affecting nearly 5% of adults and 8% of children (Sicherer and Sampson, 2014; Lopes and Sicherer, 2020). The incidence is still increasing. Foods, such as hen's eggs, cow's milk, and peanuts, are the major culprits causing life-threatening allergic reactions (Bock et al., 2007; Anagnostou, 2018). Traditional management of FA includes food avoidance and prompt identification and treatment of acute anaphylactic reactions (e.g., rapid access to epinephrine) (Gernez and Nowak-Wegrzyn, 2017). However, accidental allergen exposure is difficult to avoid, and strict diet avoidance would significantly lower the quality of life and result in several adverse outcomes such as nutritional deficiencies and psychological disorders. Current pharmaceutical agents provide only instant relief from allergies without addressing their fundamental causes. Many individuals may experience relapses of allergic reactions. Therefore, there is a need for effective and rational therapies for re-establishing tolerance to symptom-eliciting foods.

Currently, allergen-specific immunotherapy (AIT) is the only etiology-based treatment with the potential capacity for long term modification of allergic diseases (Krishna and Huissoon, 2011; Mousallem and Burks, 2012; Pajno et al., 2017). Emerging AIT strategies include subcutaneous immunotherapy (SCIT), epicutaneous immunotherapy (EPIT), sublingual immunotherapy (SLIT), and oral immunotherapy (OIT) (Nurmatov et al., 2017). Several studies have demonstrated that OIT could effectively induce short-term hyposensitization (an increased reaction threshold to food allergen during treatment) or desensitization in most enrolled subjects (with average desensitization rates of 80%–85%). Also, a minority of subjects have shown sustained unresponsiveness (SU, an absence of allergic symptoms upon allergen re-exposure weeks to years after treatment ends) (Leung et al., 2003; Akdis and Akdis, 2014; Jones et al., 2016; Nurmatov et al., 2017; Nachshon et al., 2018; Nagakura et al., 2018; The PALISADE Group of Clinical Investigators, 2018; Kim EH et al., 2020). The term SU is often regarded as a surrogate for permanent tolerance. In OIT, a dose of natural or processed food is orally administered in a gradually increasing manner to establish permanent tolerance. Protocols vary, but most include an initial rapid dose escalation phase, followed by a

✉ Zhongwei LIU, medicalman@163.com
Haitao ZHU, https://orcid.org/0000-0003-0867-1354
Zhongwei LIU, https://orcid.org/0000-0002-5548-5450

Received Jan. 26, 2022; Revision accepted May 10, 2022;
Crosschecked July 7, 2022

buildup phase, and then a maintenance phase. Several questions remain unsolved, such as the optimal dose of the offending food-allergen at each stage, the time course of the maintenance phase, and the sustainability of the desensitization process. The first controlled OIT trial for FA was reported by Patriarca et al. (1998). In the past decade, OIT has emerged as one of the most promising therapies for FA, with satisfactory therapeutic efficacy in advanced phase 3 trials (Nowak-Węgrzyn and Albin, 2015). Currently, the US Food and Drug Administration (FDA) has approved peanut allergen powder (Palforzia) for clinical application as an OIT remedy to gradually desensitize patients with a confirmed peanut allergy (The Medical Letter, 2020). Ideally, OIT aims to alleviate an allergic response by suppressing inflammatory effector cells and inducing the reinstatement of immune tolerance toward food allergens. A desensitization state is frequently induced by regular intake of the allergenic food following a schedule for conferring protection from allergic reactions. Oral tolerance is considered achieved if the food antigens can be ingested without inducing any allergic symptoms or adverse reactions despite a prolonged period of food avoidance. In general, oral food challenges (OFCs), especially double-blind placebo-controlled food challenges (DBPCFCs), are regarded as the gold standard for FA diagnosis and immune-status assessment (Bock et al., 1988; Rolinck-Werninghaus et al., 2005; Nowak-Węgrzyn and Albin, 2015). However, OFCs are time-consuming and expensive, and have a potential risk of causing anaphylactic reactions, making them unsuitable for large-scale clinical applications during OIT. Moreover, the processes of immune induction and tolerance reinstatement in OIT are complicated; OFCs are probably unable to fully reflect the immunologic status of the participants receiving OIT. Thus, biomarkers in OIT are needed for easier and more efficient clinical assessment. Ideally, biomarkers would be used to (1) accurately predict treatment response (limited remission, transient state of desensitization, or SU), (2) effectively identify risks of severe reaction, and (3) timely monitor therapeutic efficiency. To date, investigators are still searching for such ideal biomarkers.

2 Immune mechanisms involved in OIT

Oral tolerance is a state of local and systemic unresponsiveness to food proteins. After ingestion, goblet cells, microfold cells (M cells), and intestinal resident macrophages sample luminal food antigens and deliver them to lamina propria (LP) conventional dendritic cells (cDCs). Typically, cDCs ($CD11c^+$ $CD11b^+$ $MHCII^+$ cells) comprise two predominant subsets: (1) $CD103^+$ $CX3CR1^-$ DCs, which imprint gut homing on lymphocytes and induce development of regulatory T cells (Tregs), and (2) $CX3CR1^+$ DCs (with macrophage features), which facilitate tumor necrosis factor-α (TNF-α) production, colitis, and T helper 17 (Th17) cell development. Oral tolerance is initiated by $CD103^+$ DCs that capture antigens in the LP and subsequently migrate into the draining lymph nodes, where they foster differentiation of naive $CD4^+$ T cells into Tregs (Fig. 1). In addition to Tregs, T cell anergy, regulatory B cells (Bregs), host–microbe homeostasis and the functional intestinal barrier contribute significantly to the development of oral tolerance.

Although the mechanisms of OIT are not well understood, some investigations have indicated shared pathways between oral tolerance and OIT (Burks et al., 2012; Burton et al., 2014b; Nozawa et al., 2014; Escudero et al., 2015; Smaldini et al., 2015; Perezábad et al., 2017; Thota et al., 2017; Nachshon et al., 2018; Dreskin et al., 2019; Luce et al., 2020). During the initial phase of OIT, repeated exposure to a low-dose food antigen has direct effects on mast cells (MCs) and basophils, including immunoglobulin E (IgE) endocytosis and actin rearrangement, rendering these effector cells hyporesponsive to the culprit allergen. At an early stage of this phase, increased levels of specific IgE (sIgE) are commonly observed after the first allergen exposure, probably reinforcing B-cell pathogenic activities and creating an inhibitory milieu that hampers early development of Tregs. Subsequent chronic stimulation of allergen-specific Th2 cells with increasing doses of food allergen results in a counter-regulatory immune response. This presents as decreased Th2 activity and clonal expansion, increased expansion of interleukin-10 (IL-10) producing $CD4^+$ T cells, and elevated production of sIgG. Considering that allergen-specific Th2 cells are at the core of the allergic process in FA individuals, changes in the quantity and polarization of allergen-specific $CD4^+$ cells seem to be a crucial contributor to the effectiveness of OIT. Generally, the anti-inflammatory cytokine IL-10 is considered an important factor for the induction and maintenance of peripheral tolerance to allergens.

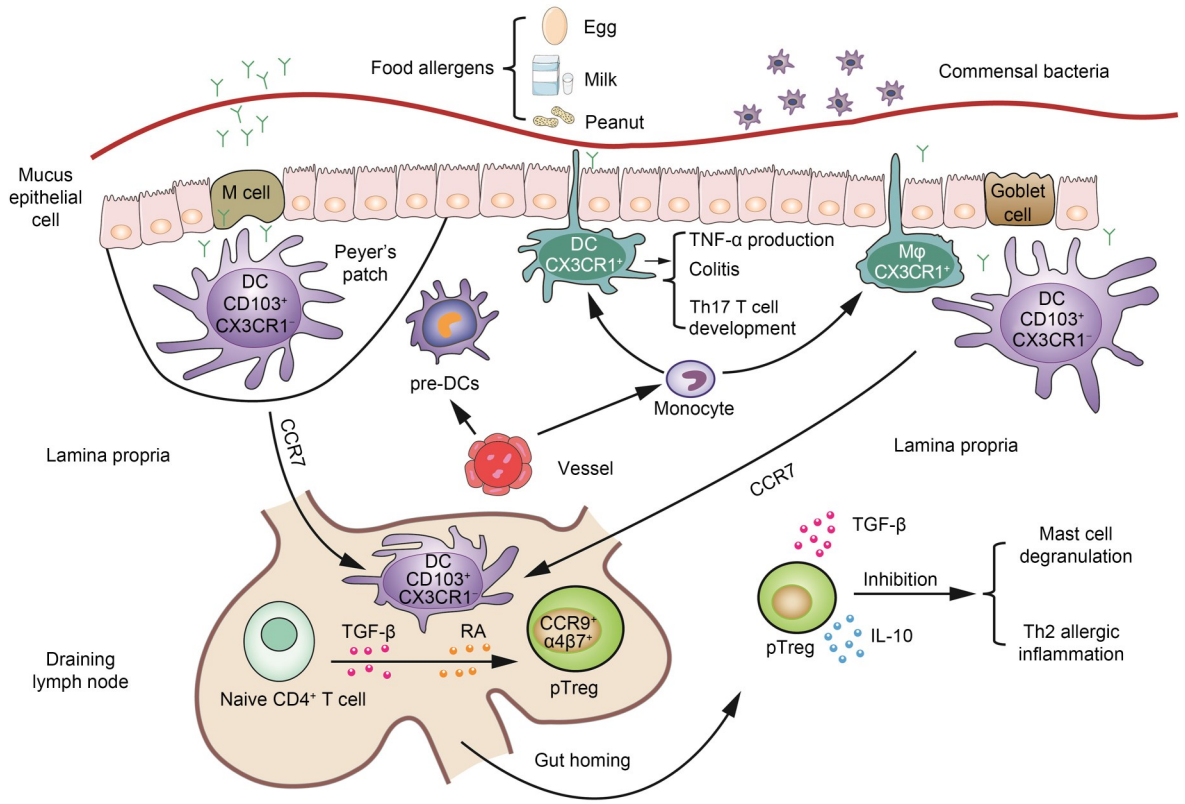

Fig. 1 Schematic diagram of immune tolerance to food antigens. Under physiological conditions, ingestion of a protein antigen can induce oral tolerance, which is mediated in part by a subset of intestinal dendritic cells (DCs) that promote the development of regulatory T cells (Tregs). Blood pre-DCs populate the lamina propria, Peyer's patch, and draining lymph nodes, and differentiate into conventional/classical DCs (cDCs). The lamina propria contains a large population of cDCs (CD11c$^+$ CD11b$^+$ MHCII$^+$ cells) comprising two predominant subsets: (1) CD103$^+$ CX3CR1$^-$ DCs, which imprint gut homing on lymphocytes and induce development of Tregs; and (2) CX3CR1$^+$ DCs (with macrophage features), which facilitate tumor necrosis factor-α (TNF-α) production, colitis, and Th17 T cell development. During immunologic tolerance, several cell types are involved in antigen uptake: goblet cells, microfold cells (M cells), intestinal epithelial cells, and CX3CR1$^+$ macrophages (CX3CR1$^+$ Mφ). After being activated by the antigen, the cDCs (also called migratory CDs) migrate into the draining lymph nodes in a C–C chemokine receptor 7 (CCR7)-dependent manner, and then foster naive CD4$^+$ T cell to differentiate into peripheral Tregs (pTregs) through epithelial cell-derived transforming growth factor-β (TGF-β) and retinoic acid (RA). The induction and development of Tregs can further prevent mast cell degranulation and Th2-dependent allergic inflammation. IL-10, interleukin-10.

Notably, the Bregs harboring immunosuppressive capacities also facilitate IL-10 production during tolerance induction (van de Veen et al., 2016). IL-10 is an important factor for IgG4 induction (Satoguina et al., 2005). During this phase, such immunologic changes are largely contingent upon continued OIT, otherwise the clinical benefits of OIT may be reduced or lost when dosing is interrupted. A possible reason is that a large population of allergen-specific CD4$^+$ T cells still remain at this point. Successful immunotherapy may induce such CD4$^+$ T cells to shift toward an "anergic" Th2 T-cell phenotype (Ryan et al., 2016). Besides, once a specific threshold of activation is achieved during chronic inflammation, IL-10- and cytotoxic T-lymphocyte antigen-4 (CTLA-4)-producing Th2 inhibitory (Th2i) cells can develop directly from nonsuppressive Th2 effectors (Altin et al., 2012). The consolidation phase begins when threshold values of activation are reached, which probably induces a selective T-cell exhaustion response away from the pro-allergic Th2 response. Prolonged stimulation during the maintenance phase can enhance epigenetic modification of the forkhead box protein 3 (*FOXP3*) locus during Tregs differentiation (Syed et al., 2014), leading to direct consequences associated with SU. The inducible Treg is considered a central mediator in the pathogenesis of FA and the acquisition of oral tolerance to food antigens (Shreffler et al., 2009; Vonk

et al., 2017; de Quiros et al., 2018). SU achievement and maintenance also require the sustained suppression or deletion of pathogenic Th2 cells, as well as the longevity and immunomodulatory function of the induced B cell memory response. The relative importance of these mechanisms and their kinetics need to be further investigated.

In short, successful OIT has a wide range of effects on many components of the host immune system. The major outcomes include: (1) decreased basophil and MC reactivity; (2) changes in allergen-specific antibodies characterized by an initial increase followed by a decrease in sIgE and a gradual increase in sIgG; and (3) altered allergen-specific T- and B-cell responses (e.g., generation of inducible Tregs). In addition, local immunity in the gut needs to be investigated further, particularly with respect to several factors involved in antigen uptake, processing, and response at the site of administration during OIT. The associated influencing factors include epithelial cells, innate lymphoid cells (ILCs), DCs, intestinal flora, and metabolites. Together with existing advances, a better, unified understanding of the complex interplay among the molecular, cellular, and humoral changes throughout the whole OIT process will be beneficial in identifying more promising OIT biomarkers in the future.

3 Functional immunological tests

3.1 Skin prick test

The skin prick test (SPT) is the most common and popular method for allergic phenotype detection. Since the first publication on SPT by Ebruster (1959), it has become the first choice in the diagnostic workup for IgE-mediated allergic diseases (mainly type I hypersensitivity reactions). This detection method is convenient and reliable, and has low cost and minimal invasiveness (Uranüs et al., 1990; Frati et al., 2018). It usually has an excellent positive predictive value (PPV) ranging from 95% to 100% (Frati et al., 2018). SPT has additional advantages of enabling the testing of multiple allergens in a matter of minutes, offering a visual indication of sensitivity, and detecting some less common allergens (e.g., certain medications). SPT rarely triggers a severe allergic reaction; anaphylactic events are more likely to occur in subjects with a history of severe reactions.

SPT is conducted by pricking the skin, usually in the forearm, with a lancet (fine needle) for delivering a trace amount of allergen extract. In a sensitized host, the relevant allergen binds sIgE, thereby cross-linking high-affinity IgE receptors (FcεRIs) on MCs, leading to degranulation of mastocytes and then the release of histamine and other mediators which generate sizeable wheals. Generally, a positive SPT is recorded as a mean wheal diameter of >3 mm (Eigenmann and Sampson, 1998; Patel and Saltoun, 2019). However, in a large-scale study of children (n=467) who underwent OFCs, it was found that food-specific SPT wheal diameters of >8 mm (to cow's milk), >7 mm (to egg), and >8 mm (to peanut) were highly and correctly predictive of systemic responses (100% specificity) to each allergen (Sporik et al., 2000). The cut-offs may vary with the age of participants, the degree of cooking, and the type of allergens (commercial extract or raw food) (Komata et al., 2007; Nowak-Wegrzyn et al., 2008; Järvinen and Sicherer, 2012; Cuomo et al., 2017). Therefore, the relevance of such sensitivity to allergen extracts should be carefully interpreted in the light of the clinical history and situation, since many SPTs may yield false positives.

In an egg OIT study (Vickery et al., 2010), the wheal diameter of the OIT group decreased significantly from a median of 10 mm at baseline to 2.5 mm at the time of OFC. In contrast, there was no significant change in the placebo group. Similar SPT results have been reported in several OITs with different food allergens (Varshney et al., 2011; Nowak-Węgrzyn et al., 2019; Moraly et al., 2020). Overall, successful OIT is closely associated with reduced SPT reactivity after treatment, and a smaller SPT diameter at initiation of OIT may be predictive of better outcomes. It is considered that the SPT probably acts as an "in vivo" test for assessing the reactivity of sIgE-activated MCs and basophils. However, SPTs do not enable a valid distinction between sensitized but tolerant individuals and clinically allergic patients. SPT seems to be suitable for the general assessment of desensitization, but unsuitable for the accurate prediction of OIT directions and outcomes. SPT may be a useful marker for monitoring the immune response during therapy.

3.2 Basophil activation test

Basophils are the essential effector cells involved in initiating the innate immune response to allergen

exposure or parasite infection. They constitute about 1% or less of circulating leukocytes. Similar to MCs, basophils express FcεRI on cell surfaces. Cross-linking of FcεRI and food allergens triggers basophil activation, and initiates subsequent intracellular signaling events that lead to the production of granule contents (histamine, proteoglycans), lipid-derived mediators (leukotrienes), and cytokines (Sarfati et al., 2015). Several studies have demonstrated a good general agreement between the expression of basophil activation markers and the time course of clinical manifestations or the development of systemic reactions in allergic patients (Gober et al., 2007; Commins et al., 2014; Santos et al., 2014; Ruinemans-Koerts et al., 2019).

The basophil activation test (BAT), also called a flow-cytometric allergen stimulation test (FAST), is a functional assay in which the expression of activation biomarkers (e.g., CD63 and CD203c) is measured on the surface of basophils following allergen stimulation (Sanz et al., 2002). A BAT can be considered an ex vivo OFC in a test tube. Typically, basophil activation is assessed by plotting a response curve to increasing antigen doses. Based on the dose response, two indicators of basophil reactivity and basophil sensitivity (50% effective concentration (EC_{50}) or similar) have been extensively adopted and applied. In general, basophil reactivity refers to the proportion of basophils that express CD63 (percentage of $CD63^+$ basophils) compared to the controls (Santos et al., 2015a, 2021). Basophil sensitivity is measured as the median effective dose (ED_{50}) or basophile allergen threshold sensitivity (CD-sens), expressed as the inverse of the EC_{50} multiplied by 100 ($CD\text{-}sens=1/EC_{50}\times100$) (Glaumann et al., 2012; Santos et al., 2015a; Santos and Lack, 2016).

CD63 is a lysosomal-associated membrane protein (LAMP) located on the membrane of secretory lysosomes in resting basophils. When the granules fuse with the plasmatic membrane of basophils during degranulation, CD63 begins to express on the cell surface (Amano et al., 2001). CD63 expression is directly and strongly correlated with histamine release in the cell supernatant. CD203c, a surface marker unique to basophils and MCs, is already expressed on resting cells and can be up-regulated by IL-13 stimulation. After activation, CD203c is up-regulated slightly earlier than CD63. In general, CD107a and CD107b co-localize with CD63 in secretory lysozymes, whereas CD164 and CD13 co-localize with CD203c in vesicles.

Compared with SPTs, BATs are better for distinguishing between allergic and tolerant individuals (Ocmant et al., 2009; Song et al., 2015). In the first large-scale study assessing tests for the diagnosis of peanut allergy (Santos et al., 2014), BAT optimal diagnostic cutoffs showed 98% sensitivity, 96% specificity, and 97% accuracy, and also allowed a reduction of about 66.6% in the number of OFCs required. Moreover, the use of BATs in a two-step sequential approach (a combination of a BAT with another test of SPT or sIgE) could further improve diagnostic accuracy in children with an equivocal SPT and sIgE to peanut and its components. In recent years, subsequent studies have extensively assessed the performance of BATs in the diagnosis of allergy to different foods, including cow's milk (Rubio et al., 2011; Ruinemans-Koerts et al., 2019; Kim YH et al, 2020; Nucera et al., 2021), egg (Kim YH et al., 2020; Nucera et al., 2021), fish (Imakiire et al., 2020), wheat (Chinuki et al., 2012), hazelnut (Cucu et al., 2012), and shrimp (Song et al., 2015; Wai et al., 2021). The BAT seems to be a reliable and cost-effective tool in the management of pediatric FA. With high diagnostic accuracy, the BAT shows advantages when SPT and sIgE are equivocal and is a necessity for OFCs. The high specificity of BAT confers a high degree of certainty and consistency in confirming FA diagnosis (Kim YH et al., 2020; Santos et al., 2020, 2021). More importantly, the BAT is a useful tool to determine whether an OFC can be safely undertaken. Using a large cohort of well-characterized patients, Santos et al. (2020) first analyzed the utility of different indicators, including BAT, SPT, peanut-sIgE, *Arachis hypogaea* 2 (Ara h 2)-sIgE, and peanut-sIgG4, in relation to peanut allergy status, severity, and threshold dose of allergic reactions during OFC (Santos et al., 2020). The data confirmed that the BAT is a superior biomarker for peanut allergy with high specificity (98.7%). Also, the BAT was the best predictor for identifying life-threatening allergic reactions (with 97% specificity and 100% sensitivity) and the threshold dose of reactivity to peanut (with 91% specificity and 95% sensitivity) during OFC. The BAT results appear to be unaffected by a relatively long-term (about three months) intake of antiallergic drugs (antihistamines) in FA patients (Nucera et al., 2021).

Apart from investigating a patient's allergic status at a given time point, BATs can be used to monitor clinical response, to evaluate the natural resolution of FA and to assess immunologic changes during immunotherapy. Of note, decreased basophil reactivity and sensitivity related to desensitization have been observed in OITs with different culprit foods, such as peanut (Jones et al., 2009; Marrs et al., 2015; Patil et al., 2019; Tsai et al., 2020), egg (Kim EH et al., 2020), and milk (Frischmeyer-Guerrerio et al., 2017; Vonk et al., 2019). Loss of basophil activation may appear as one of the earliest readouts observed at an immunological level during OIT. Significantly, suppression of CD63 and/or CD203c expression of basophils was observed during the initial phases of OIT (Gorelik et al., 2015). This is consistent with the early immunologic changes during OIT. In a peanut OIT trial (Patil et al., 2019), an early decrease in basophil sensitivity to Ara h 2 within the first three months was closely correlated with SU after treatment. However, after four weeks of peanut avoidance (cessation of OIT), the basophil reactivity rebounded in subjects with transient desensitization. A recent peanut OIT trial also demonstrated that combined assessments of BAT and sIgE might be helpful in predicting therapeutic outcomes and differentiating between transient desensitization and an SU state (Tsai et al., 2020). Therefore, the combination of BAT and other tests or biomarkers may be a useful, less-invasive, and safe strategy for monitoring OIT efficacy. Notably, for large-scale clinical applications of BAT, it still requires analytical and clinical validation, standardization of procedures, and quality assurance to ensure the reproducibility and reliability of results. Currently, efforts are being made to establish a platform that can be used by laboratories in Europe and the USA for quality assurance and certification.

3.3 Mast cell reactivity

MCs are located in skin and gastrointestinal and respiratory tracts, where the host immune system frequently interacts with the external environment. They serve as tissue resident cells and function as the first line of defense against invading pathogens (Crivellato et al., 2010; Kubo, 2018).

Similarly to basophils, MCs play important roles in allergic processing. All these cells express FcεRI receptors, which are crucial for allergen recognition and successive cell activation/degranulation. A study in an FA mouse model indicated that mucosal MCs may amplify the allergic response to ingested peanut and suppress the induction of peanut-specific Tregs (Burton et al., 2014a). Strikingly, deletion of spleen tyrosine kinase (Syk, FcεRI signaling kinase) solely from MCs was sufficient to restore Tregs induction. Accumulation of MCs in the local mucosa of gastrointestinal tracts is often observed during food-antigen-induced allergic diarrhea in murine models (Kurashima et al., 2007). Furthermore, duodenal biopsies from FA patients have shown enhanced expression of MC-associated transcripts when compared with controls (Chen et al., 2015). All these findings indicate that activated and degranulated MCs are essential pathological elements for the development of allergic reactions. Recent data also demonstrated that MC progenitors could become activated by IgE cross-linking, and may contribute to the pathology associated with acute allergic airway inflammation via the production of IL-13 (Méndez-Enríquez et al., 2022). In contrast, some studies have indicated that the MCs may exert immunoregulatory functions during long-term stimulation, which is confirmed by the production of regulatory cytokines, such as IL-2 and IL-10 (Leveson-Gower et al., 2013; Morita et al., 2015). The most probable cause is that continuous stimulation by the antigen-IgE complex can modulate FcεRI signal transduction, resulting in a functional change in MCs (Ang et al., 2016; Salamon et al., 2017). By developing a clinically relevant OIT murine model, Takasato et al. (2021) further demonstrated this innovative mechanistic immunological process in the intestinal mucosa upon successful OIT. In their study, ovalbumin (OVA) OIT could effectively induce oral unresponsiveness against allergen-induced allergic diarrhea in mice by conversion of activated pathogenic MCs to desensitized regulatory MCs. That is, OIT prompted desensitized regulatory MCs to produce IL-2 for the expansion of Treg populations and IL-10 for suppression of allergic responses, whereas the production of pathogenic Th2 cytokine was inhibited. Also, the desensitization process may modulate the activation of MCs, leading directly to enhanced induction of Tregs and promotion of clinical allergic unresponsiveness (Takasato et al., 2021). These findings provide new insights into the pathogenic and beneficial aspects of MCs, which can be manipulated by an appropriate form of OIT to regulate allergic responses.

Although MC activation tests (Bahri et al., 2018; Santos et al., 2018) and serologic determination of activation products (e.g., MC protease-1) (Burton et al., 2014a) provide favorable diagnostic performance, these tests may not perfectly recapitulate MC actions in situ since cell responses to micro-environmental cues may change MC phenotype and function. On the other hand, the MCs may also contribute to immunological transition from allergic promoter to suppressor during the desensitization process. Current available tests seem not to accurately reflect activation and functional changes of MCs during FA and OIT processes. Traditionally, since basophils are more accessible than MCs for ex vivo studies, most researchers prefer to apply the BAT as an assessment of therapeutic efficacy during OIT. However, MCs, especially mucosal MCs (MMCs) localized in the intestinal mucosa, play a central role in the development of FA. The MMCs are a distinctly different population from connective tissue MCs (Nakano and Kitaura, 2022), and are gradually emerging as an attractive target for therapeutic intervention in food-induced disorders. The functions of MMCs are still poorly understood. Recent evidence suggests orally desensitized MCs (regulatory MCs) seem to form a regulatory network with Tregs for allergy treatment, potentially providing a promising target or biomarker for successful OIT (Takasato et al., 2021). Moreover, transcriptome and proteome analyses suggest that human MCs are not so different from mouse MCs (Plum et al., 2020). Thus, a mouse model of OIT is an excellent alternative at present for the detailed and in-depth study of human MMC without excessive technical and ethical difficulties. Research focusing on MCs will further improve the treatment of FA. With advances in the characterization of MC subtypes, molecular and regulatory mechanisms of MC expansion, differentiation, and activation, as well as in vivo living cell imaging techniques and humanized models, it should be possible to introduce more promising clinical indicators for prediction of AIT outcomes. Such progress will be beneficial for identifying the exact roles of innate immune cells in the development of an allergic or oral tolerance response.

4 B cell responses

B cells play an important role both in the development of allergen sensitization and induction of allergen tolerance. The contribution of B cells to such processes is associated with antibody production, interplay with other cell types, secretion of various cytokines, and expression of surface molecules. In the sensitization of FA, B cells respond to allergic stimuli primarily by producing high-affinity specific antibodies which serve as hallmarks of an adaptive immune response (Calderon et al., 2010; Shakoor et al., 2016; Smeekens and Kulis, 2020). During OIT, major changes of humoral response are associated with increased food-specific IgG and IgA, as well as initially increased, but subsequently decreased, food-specific IgE. The involvement of B cells themselves in tolerance induction is not as well understood as that of T cells, but a few studies focused mainly on the Bregs have implicated the role of B cells in AIT.

4.1 Immunoglobulin quantification

4.1.1 Immunoglobulin E (IgE)

Testing for IgE sensitization is the cornerstone of diagnostic evaluation in suspected allergic conditions (Ansotegui et al., 2020). The level of sIgE may serve as a useful tool for estimating the probability of immune reaction to raw food, food extracts, or molecular components (Hao et al., 2016). However, a high sIgE level does not directly correlate with the severity of the reaction. The epitopes (regions on the allergen) to which IgE binds seem to have more clinical relevance to factors such as patient heterogeneity and clinical reactivity. IgE epitope mapping is usually conducted using a peptide microarray immunoassay. Determination of epitope recognition provides an additional tool in predicting the phenotype of milk allergy (Sackesen et al., 2019), as well as identifying symptomatic peanut allergy, especially in children with peanut sIgE below diagnostic decision levels (Beyer et al., 2003). A linear epitope analysis of sIgE in peanut-sensitized patients revealed not only variability in the number of bound epitopes (Shreffler et al., 2005), but also a positive association with reaction severity (Flinterman et al., 2008). Patients with greater epitope diversity (number of epitopes recognized) appear to be more sensitive than those with the lower diversity (Flinterman et al., 2008). The qualitative aspects of sIgE, such as clonality, epitope specificity and diversity, and post-translational modifications, may also play a decisive role in the allergic process. In a prospective cohort study of infants at high risk for allergy,

an algorithm combining epitope sIgE with peanut sIgE outperformed different clinically relevant IgE cutoffs, predicting allergy status on an "unseen" set of patients at the end point (Suprun et al., 2020).

Several studies have shown that food sIgE is up-regulated after the onset of OIT and decreases thereafter over the course of therapy (Varshney et al., 2011; Anagnostou et al., 2014; The PALISADE Group of Clinical Investigators, 2018; Hourihane et al., 2020). The level of component-specific IgE also seems to follow trends similar to those of food-specific IgE. In a pilot clinical trial of peanut OIT, the change over time in total peanut sIgE levels was paralleled by similar changes in IgE specific for Ara h 1 and Ara h 2 (Vickery et al., 2014). However, the levels of other component-sIgEs (specific for Ara h 3, Ara h 8, and Ara h 9) did not change at different time points, indicating that IgE binding to major allergen components is an important target during OIT. During peanut OIT, in subjects with peanut sIgE of ⩾35 kIU/L, a combined model of IgE binding to epitopes 1, 5, and 6 (linear epitopes of Ara h 2) with peanut-sIgE was highly predictive of attainment of SU after OIT (Dreskin et al., 2019). In a cow's milk study (Savilahti et al., 2014b), children who attained desensitization showed decreased epitope sIgE and increased epitope sIgG4 following therapy. However, in subjects with OIT discontinuation, the reduced quantity/affinity of epitope sIgE was reversed, and there was a broader diversity of IgE and IgG4 binding. Further study demonstrated that as a predictive model of SU, IgE-binding epitopes alone performed significantly better than models using standard serum component sIgE (Suárez-Fariñas et al., 2019). Therefore, measuring the epitope repertoire may be a reliable approach to predict the probability of SU and the threshold of clinical reactivity after OIT.

In addition, the ratio of sIgE to total IgE has been reported to be more predictive for OFC outcomes than sIgE alone (Gupta et al., 2014). The ratio has been investigated as another potential biomarker for OIT (Patil et al., 2020). However, such indicators were examined in a small cohort and need to be further verified with more trials involving larger sample sizes.

4.1.2 Immunoglobulin G (IgG)

Food sIgG antibody often rises in the setting of naturally resolving FAs, but in unsensitized individuals, IgG production to ingested foods is in the normal range (Schwarz et al., 2016). This raises the possibility that IgG may serve as an indicator for allergy and tolerance evaluation. As OIT continues and higher doses of antigen are administered, the level of allergen sIgG, particularly IgG4, gradually increases in a dose-dependent manner within weeks to months (Jones et al., 2009; Burks et al., 2012; Vickery et al., 2014). In general, the IgG4 level increases more than 10-fold from the baseline value and remains elevated, even after several years of OIT. The phenomenon of an initial up-regulated sIgG4 level followed by a later plateau phase has been reported in several OIT trials with different foods including peanut, egg, and milk (Jones et al., 2009; Burks et al., 2012; Nozawa et al., 2014; Tang et al., 2015; Wright et al., 2016; Vickery et al., 2017; Nagakura et al., 2021). In the mouse model of FA, allergen sIgG can facilitate tolerance restoration and sustainability, favoring expansion of $Foxp3^+$ Tregs along with suppression of existing Th2 and IgE responses (Burton et al., 2018). Other studies have also demonstrated the ability of IgG to suppress IL-4 secretion and shift the Tregs/Th2 balance (Burton et al., 2014b, 2018). Increases in sIgG4 antibody levels, with or without decreases in IgE levels, are directly associated with successful immunotherapy, and IgG blocking activity is correlated with SU.

After peanut OIT, increases in Ara h 2 sIgG4 and total IgG levels coincide with the increase in the frequency of memory B cells and plasma cells during the initiation phase of OIT. This suggests that these cells provide a clonal contribution to the functionally suppressive antibody. This viewpoint is further supported by the appearance of somatic hypermutation in allergen sIgG4 (Hoh et al., 2016) and the observation of global epitope-specific shifts from IgE to IgG (induction of new Ara h 2 sIgG4) (Vickery et al., 2013). The up-regulated secretion of IgG4 also may be related to IL-10 production by Tregs and Bregs (Satoguina et al., 2005; van de Veen et al., 2013). Typically, serum sIgG4 acts as a blocking antibody isotype to compete with sIgE for allergen binding, thereby suppressing allergen-IgE complex formation (namely allergen neutralization) and preventing MC and basophil degranulation. Also, IgG bound to the surface receptor FcgRIIb can trigger inhibitory signaling with IgE and IgG cross-linking (Cassard et al., 2012; Burton et al., 2014b, 2018). Coaggregation of FcεRI with FcgRIIb further suppresses the adaptive allergic responses by preventing

both degranulation and downstream cytokine synthesis (e.g., IL-4).

In recent peanut OITs, plasma from subjects with active OIT and peanut tolerance (peanut-sensitized, but tolerant) significantly inhibited basophil activation. Furthermore, depletion of IgG4 reversely restored the peanut-induced basophil/MC activation (Santos et al., 2015b; Orgel et al., 2019), suggesting that BATs may be useful for assessing functional changes in IgG blocking functionality during OIT. A comprehensive assessment of the magnitude and blocking-activity of sIgG4 may greatly improve the predictive values of this immunological parameter in relation to OIT response. During OIT, a rapid increase of IgG4-binding epitopes and a slow decrease in IgE amounts and/or binding epitopes have been often observed in participants who reached desensitization (Vickery et al., 2013; Savilahti et al., 2014b; Martínez-Botas et al., 2015). Therefore, the development of higher sIgG/IgE ratios may be more likely to predict lasting tolerance than the absolute quantity of IgG4 (Wright et al., 2016; Chinthrajah et al., 2019). The practicability of such ratios for predictive modeling application needs further assessment in more clinical trials.

Other subclasses of the IgG family, including IgG1, IgG2, and IgG3, have also been investigated and quantified in the course of AIT (Sugimoto et al., 2016; Heeringa et al., 2020). During OIT, serum levels of these IgGs change in a manner similar to that of IgG4 (Savilahti et al., 2014a; Sugimoto et al., 2016). Significant high-fold increases in egg sIgG1 have been observed in responders with sustained desensitization, rendering the characteristic changes of IgG1 level as a potential biomarker for the prediction of a positive clinical response to OIT (Sugimoto et al., 2016). However, there have been few such studies, and each included only a few patients, and the precise responses of such sIgG subclasses during OIT still remain to be clarified.

4.1.3 Immunoglobulin A (IgA)

IgA, as the major secretory immunoglobulin isotype found at mucosal surfaces, plays a key role in immune exclusion. Repeated mucosal allergen exposure in OIT has the potential to induce the production of allergen sIgA and secretory IgA. The secretory IgA has the ability to prevent allergens from accessing the systemic immune system and suppressing inflammatory responses. Moreover, for maintenance of intestinal homeostasis, secretory IgA and Tregs regulate each other in a double negative-feedback loop, which can be transferred maternally through the entero-mammary axis (Tsuji et al., 2009; Feng et al., 2011; Ramanan et al., 2020).

IgA deficiency may increase the risk of FA and other allergic manifestations (e.g., allergic rhinitis and atopic eczema) in childhood (Lúðvíksson et al., 2005; Janzi et al., 2009). During egg OIT, increased levels of serum egg-white sIgA and sIgA2 were associated with the clinical response to immunotherapy (Wright et al., 2016). Compared to sufferers with transient desensitization, subjects with SU showed higher levels of allergen sIgA. In contrast, a previous study suggested that serum sIgA appeared not to be associated with induced or natural tolerance, since it remained unchanged after egg OIT or avoidance (Vazquez-Ortiz et al., 2013). Interestingly, peanut sublingual immunotherapy demonstrated that salivary IgA might be useful in determining therapeutic efficacy and could serve as a potential biomarker to follow throughout therapy (Kulis et al., 2012). The precise role of IgA in FA development and tolerance induction is still poorly understood. For OIT studies, the existing findings are not sufficient to determine whether IgA is an efficient biomarker for predicting treatment response and SU. Note, however, the thorough elucidation of key pathways in oral tolerance at mucosal sites and emerging tools for IgA detection (e.g., high affinity recombinant IgA-binding peptide probes and enzyme immunoassays for salivary IgA) (Planchais and Mouquet, 2020; Costantini et al., 2022) will likely render IgA a complementary indicator for OIT efficacy.

4.2 B cells

In peanut allergic patients, allergen-specific B cells can be found in circulation at very low frequencies of 0.0097% (Ara h 1) or 0.029% (Ara h 2) of B cells at baseline (Hoh et al., 2016). Approximately three-fold increases in the frequencies of such circulating allergen-specific B cells were observed in response to OIT (Patil et al., 2015; Hoh et al., 2016). The allergen-specific B cells were primarily of the memory phenotype, and predominantly expressed somatically mutated antibodies of IgG and IgA which recognize a variety of conformational or linear epitopes (Hoh et al., 2016; Boonpiyathad et al., 2019).

Notably, during OIT, increasing somatic mutations of IgG4 members of a peanut-specific clone were detected, whereas IgE-expressing clone members showed no change in mutation. This indicates that the ongoing somatic mutation of IgG4-expressing B cells might improve OIT efficacy, possibly by enhancing IgG4 affinity for the allergen.

Bregs are a subset of IL-10-producing B cells involved in the maintenance of tolerance and the restoration of homeostasis following inflammation. IL-10 serves as an essential activator for inducing an immunoregulatory phenotype in B cells (Stanic et al., 2015). IL-10-overexpressing B cells regulate the responses in both innate and adaptive immunity via various pathways (Stanic et al., 2015), such as (1) suppressing DC maturation, T effector cell proliferation, and IgE production, (2) promoting production of anti-inflammatory factors, and (3) lowering production of pro-inflammatory cytokines. In addition to IL-10, secretion of transforming growth factor-β (TGF-β) and IL-35 may contribute to Breg-mediated immunosuppression. There is still a lack of distinctive phenotypic or lineage markers and specific cytokine profiles that can accurately characterize Bregs. One study indicated that human-inducible IL-10-secreting Bregs ($CD73^{-}CD25^{+}CD71^{+}$ B cells, Br1 cells) take an active part in peripheral allergen tolerance in beekeepers (van de Veen et al., 2013). An increased frequency of Br1 cells was observed in bee venom allergic subjects receiving AIT. The inducible Br1 cells could potently suppress antigen-specific $CD4^{+}$ T-cell proliferation and produce sIgG4 when they switched to plasma cells. Allergen sIgG4 is a crucial contributor for inducing and sustaining immune tolerance during OIT. A subsequent study further demonstrated a similar immunoregulatory role for such B cells (Br1 cells and sIgG4-switched memory B cells) in allergen tolerance in both AIT patients and naturally exposed beekeepers (Boonpiyathad et al., 2017). In a casein-induced allergy model in mice, IL-10-producing $CD5^{+}$ B cells derived from mesenteric lymph nodes (MLNs) effectively inhibited the allergic response in a $Foxp3^{+}$ Tregs-dependent manner. The underlying mechanism likely involves the paracrine action of Breg-derived IL-10 on $Foxp3^{+}CD25^{+}$ T cells within MLN, possibly providing a novel therapeutic regulator for FA treatment. A recent study suggested for the first time that pollen SCIT could lead to an increase in the number of IL-10-producing Bregs ($CD19^{+}CD5^{hi}CD1d1^{+}$ and $CD19^{+}CD24^{hi}CD38^{hi}$ B cells), which was associated with an increased IgG4 level and enhanced local IgE inhibitory activity in nasal fluid (Shamji et al., 2019). The nasal IgG4-associated inhibitory activity correlated closely with the clinical response of SCIT. The study indicates the potential role of Bregs in inducing immune tolerance against aeroallergens. These findings should be investigated in other AIT models to define the capacity of Bregs to serve as candidate biomarkers for a successful AIT response.

5 T cell responses

During an allergic response, T-cell activation drives the main effector phases of sensitization (Schmidt-Weber and Blaser, 2002; Tordesillas and Berin, 2018). T-cell differentiation and activation events take place primarily through a Th2-biased response pathway initiated by epithelium-derived cytokines (Th2-inducing cytokines), such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 (Eiwegger et al., 2019; Hong et al., 2020; Nakajima et al., 2020). In contrast, immune tolerance to food antigens is mediated mainly by Tregs induction. Indeed, FA is induced by the loss of tolerance to food proteins, and is characterized by an altered balance of Treg cells and a shift to Th2-type cytokines in the intestinal lamina propria. Thus, Th2 cell activation and Tregs deficiency are vital features of allergy. The changes in the magnitude and polarization of allergen-specific T cells are likely to be key components of OIT effectiveness, emerging as biomarkers for predicting OIT outcomes.

5.1 Tregs

Tregs, as essential mediators of peripheral tolerance to self- and non-self-antigens, are critical to the induction and maintenance of oral tolerance. To a certain degree, $Foxp3^{+}$ Tregs rather than T cell anergy or depletion confer functional oral tolerance. As demonstrated in patients with immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome, dysfunction of transcription factor $Foxp3^{+}$, which drives the genetic programming of Treg cells, is closely related to an increased incidence of FA (Torgerson et al., 2007; Attias et al., 2019). In a mouse model of OVA-induced allergic diarrhea, $Foxp3^{+}$ Tregs proliferated and accumulated in the intestinal

LP during tolerance induction, and depletion of $Foxp3^+$ cells completely abolished established oral tolerance (Hadis et al., 2011). Similarly, in a model of contact hypersensitivity (CHS), transfer of $CD4^+CD25^+$ cells into invariant chain knock-out mice (deficient in $CD4^+$ T cells) prevented the CHS response and skin infiltration mediated by $CD8^+$ T cells (Dubois et al., 2003). In normal mice, in vivo depletion of $CD4^+CD25^+$ cells by antibody (clone PC61) treatment reversely impaired oral tolerance. In FA mice, intragastric administration of cow's milk proteins (OIT intervention) pre- or post-sensitization suppressed the Th2-immune response and simultaneously alleviated hypersensitivity symptoms, mainly through the elicitation of mucosal IL-10- and TGF-β-producing Tregs (Smaldini et al., 2015). Tregs are well known to be associated with the production of IL-10, TGF-β, and IL-35. IL-10 can significantly suppress sIgE production and induce sIgG4 production via Treg-B cell interaction (Jutel et al., 2003). Tregs also express suppressor molecules, such as CTLA-4 and programmed cell death-1 (PD-1), both of which contribute to immune tolerance as co-stimulation blockades. In a clinical trial (Syed et al., 2014), 23 participants underwent peanut OIT for 24 months. They were deemed to have clinical immune tolerance if they had no detectable allergic reactions to OFC after three months of treatment withdrawal. Compared to non-tolerant and control participants, immune tolerance participants showed higher numbers of antigen-induced Treg (ai-Treg) with greater suppressive function and higher levels of *FOXP3* hypomethylation. In contrast, the state of "resensitization" was correlated with increased methylation of cytosine-phosphate-guanosine (CpG) sites in the *FOXP3* locus.

Besides conventional $Foxp3^+$ Tregs, another type of Tregs named Th3 cells, also contributes a crucial role in oral tolerance. Th3 cells do not express Foxp3 or CD25 molecular, but express latency-activated peptide (LAP) on their surface. These cells produce high amounts of TGF-β and moderate amounts of IL-10, and are usually identified as TGF-β-producing $CD4^+Foxp3^-LAP^+$ T cells. In the absence of inflammation, TGF-β secretion can promote the expression of the *FOXP3* gene in activated T cells during T cell expansion, thereby inducing the differentiation of Tregs in the peripheral repertoire in the absence of thymic $CD25^+$ Tregs (Carrier et al., 2007). Therefore, Th3 cells probably act as a central mediator of peripheral immune tolerance both directly by TGF-β production and indirectly by $Foxp3^+$ Tregs induction. EPIT generates gut-homing LAP^+ Treg cells that can directly suppress systemic anaphylaxis without upstream modification of humoral or cellular immunity (Tordesillas et al., 2017). The mechanism of protection is considered a novel pathway of direct TGF-β-dependent Treg-cell suppression of MC activation.

All these data indicate that induction and expansion of Tregs might be available and crucial indicators for reflecting the state of immune tolerance during OIT. Epigenetic changes in the *FOXP3* locus appear to be the crucial factor directly regulating Treg plasticity and suppressive function. However, these findings are based on studies with small sample sizes and short follow-up periods. In future, with exploration of the precise relationships among different Treg subsets and identification of unique phenotypes, large-scale, long-term follow-up studies may further assess the clinical significance of Tregs for predicting OIT outcomes, particular SU.

5.2 Th2-type cytokines

Th2 cytokines, including IL-4, IL-5, IL-6, IL-9, IL-13, and IL-17E (IL-25), are all-important hormonal messengers responsible for several biological effects in the host immune system (Berger, 2000; Smart and Kemp, 2002; Akdis et al., 2016; Chinthrajah et al., 2016; Shik et al., 2017) (Table 1). For example, in allergic inflammation, IL-5 is associated with eosinophil recruitment. IL-4 and IL-13 are associated with induction of IgE class switch recombination, smooth muscle cell contraction, and mucus production. IL-25 is involved in mucosal immunity and inducing production of other Th2 cytokines IL-4, IL-5, and IL-13 (Chinthrajah et al., 2016). In sensitization, epithelial disruption allows increased antigen exposure and induces production/release of epithelial cytokines (e.g., IL-33, TSLP, and IL-25) which can up-regulate OX40 ligand (OX40L) on DCs. The activated DCs then promote differentiation of naive T cells into Th2 cells producing cytokines that recruit eosinophils (IL-5) and promote IgE class-switching in B cells (IL-4 and IL-13).

In OIT studies, measurements of secreted Th2 cytokines in vitro can be performed in serum or stimulated peripheral blood mononuclear cells (PBMCs). Various testing methods, including enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot

Table 1 Th2 differentiation/secreted cytokine profiles

Cytokine	Cell source	Classification	Characteristics and functionality
IL-2	T cells (mainly Th1 cells)	Common γ-chain family (IL-2 family)	Required for differentiation to Th1 and Th2 subsets Promotes proliferation of T cells, B cells, and NK cells Involved in Tregs development Augments cytokine secretion and cytolytic activity
IL-4	T cells, NK cells, eosinophils, mast cells	IL-4/IL-13 family	Promotes Th2 cell differentiation Stimulates expansion of activated B cells, T cells, and mast cells Induces class switch of antibodies; promotes IgE production
IL-6	Th2 cells, monocytes, macrophages, BMSCs	IL-6 family	Keystone cytokine in health and disease Exhibits contrasting features of proinflammatory and anti-inflammatory profiles Induces differentiation of monocytes and Tfh cells Involved in B cell maturation; induces fever and APP synthesis Regulates naive $CD4^+$ T cell differentiation Dysregulation is implicated in the onset/development of AICIDs (offering IL-6-targeted therapies, e.g., Satralizumab, Siltuximab, Tocilizumab, and Olamkicept)
IL-27	Monocytes, endothelial cells, DCs	IL-6 family	Dual immune-regulations depending on context Induces Th1 cell expansion Synergizes with IL-12 to promote production of IFN-γ by naive $CD4^+$ T cells Promotes IL-10 production and specializes Treg response Candidate antitumor agent
IL-17E (IL-25)	Epithelial cells, macrophages, mast cells, Th2 cells, eosinophils, basophils	IL-17 family	Promotes cell expansion and Th2 cytokine production (IL-4, IL-5, IL-13, and TSLP), and sustains type 2 immunity A "barrier surface" cytokine, involved in mucosal immunity Therapeutic target for treatment of severe asthma exacerbation
IL-33	Endothelial cells (e.g., HEVECs), epithelial cells, fibroblast-like cells, myofibroblasts (e.g., pericryptal fibroblasts), mast cells, DCs (in inflammatory condition)	IL-1 family	Drives early immune response against allergens and other environmental insults, necessary for Th2 cytokine production Regulates DC functions, drives and influences development of Th2, Tfh, and Treg cells A central pathway in pathogenesis of allergic inflammation (IL-33-ST2-ILC2s axis), biomarkers for defining AAD subtypes Maintenance of tissue homeostasis, repair, and remodeling
IL-31	Th2 cells, mast cells, macrophages, DCs, eosinophils	Gp130/IL-6 family	Implicated in ASDs; offers candidate therapeutic approaches (e.g., Nemolizumab) Potential pathway of inflammation in allergic and autoimmune diseases (IL-31/IL-33 axis) Recruitment of PMNs, monocytes, and T cells to inflammatory sites (by inducing chemokine production) Dual role in immunomodulation (in murine asthma models, early proinflammation Th2 response→later negative feedback response)
IL-3	T cells, NK cells, mast cells	HCs family	Supports growth and differentiation of hematopoietic cells Stimulates mast cell growth and histamine secretion
IL-5	Th2 cells, mast cells	HCs family	Stimulates B cell/eosinophil growth and differentiation Induces class switch to IgA Candidate therapeutic target for NPs, asthma, and other ADs (e.g., Mepolizumab, Reslizumab, and Benralizumab)

To be continued

Table 1 (continued)

Cytokine	Cell source	Classification	Characteristics and functionality
IL-10	T cells (mainly Th2 cells), macrophages, monocytes, DCs, neutrophils, mast cells, eosinophils, NK cells, epithelial cells, tumor cells	IL-10 family	Triggers a robust immune suppressive response (classic role) Enhances B cell survival, proliferation, and antibody production Limits damaging effects of neuroinflammation Involved in AT regulation; promotes epithelial wound healing Therapeutic manipulations in various pathologies (e.g., IBD, cancer, psoriasis, hepatitis C, RA, SLE, and asthma)
IL-13	Th2 cells (mainly), mast cell	IL-4/IL-13 family	Similar to IL-4 functions Potential pathways in allergic, autoimmune, fibrotic and neoplastic diseases (IL-4/IL-13 axes) Candidate therapeutic approaches (e.g., Dupilumab, Lebrikizumab, Anrukinzumab, Tralokinumab, and AS1517499)

IL, interleukin; Th1, T helper type 1; Tregs, regulatory T cells; NK, natural killer; IgE, immunoglobulin E; BMSCs, bone marrow stromal cells; Tfh, T follicular helper; APP, acute-phase protein; AICIDs, autoimmune and chronic inflammatory diseases; DCs, dendritic cells; IFN-γ, interferon-γ; TSLP, thymic stromal lymphopoietin; Gp130, glycoprotein 130; ILC2s, group 2 innate lymphoid cells; ST2, tumorigenicity 2 receptor (a receptor for IL-33); HEVECs, high endothelial venule endothelial cells; AAD, allergic airway disease; ASDs, autoimmune skin diseases; PMNs, polymorphonuclear leukocytes; HCs, hematopoietic cytokines; NPs, nasal polyps; ADs, allergic diseases; AT, adipose tissue; IBD, inflammatory bowel disease; RA, rheumatoid arthritis; SLE, systemic lupus erythematous.

assay (ELISpot), co-culturing T cells with DCs, tetramer analysis, and intracellular staining, have been used to detect not only Th2 response (IL-4, IL-5, IL-9, and IL-13), but also Th1 (IFNγ) and Treg (IL-10 and TGF-β) responses during AIT. Generally, AIT intervention appears to act through down-regulation of allergen-specific Th2 response or increased Th1 response, or through the induction of Tregs. In an egg OIT study (Perezábad et al., 2015), at baseline, the egg-allergic patients presented a trend toward a higher production of Th2 cytokines (IL-5 and IL-13) and significantly lower levels of IL-10 (P<0.01) after PBMC stimulation with OVA. After completing egg OIT, these subjects showed cytokine profiles of decreased production of IL-5 and IL-13, as well as higher levels of OVA-specific IL-10 (P<0.05). Cytokine variation during OIT might be a feature of the transition from allergen-specific Th2 to Treg predominance.

Several studies have indicated that Th2 cytokines (IL-5 and IL-13) decline significantly during peanut OIT (Varshney et al., 2011; Kulis et al., 2019). One study involving 39 peanut-allergics demonstrated that subjects with clinical desensitization had higher secretion of IL-10, IFN-γ, and TNF-α from PBMCs over a period of 6 to 12 months (Jones et al., 2009). However, in another study, only a transient increase in TGF-β level was observed in peanut OIT subjects (Varshney et al., 2011), and no significant change was observed in IL-10 or IFN-γ (Varshney et al., 2011; Kulis et al., 2019). Similarly, transient increases were seen in egg-induced IL-10 and TGF-β levels, and the ratio of Th2:Th1 cytokine production decreased (Vickery et al., 2010). In a study of cow's milk allergy, a marked decrease in IL-13, IL-5, and IL-10 production (statistically significant for IL-13 and IL-10) by PBMCs upon β-casein stimulation was observed in children after OIT treatment (Perezábad et al., 2017). Interestingly, IL-10 production from non-allergic donors was significantly higher (P<0.01) than that from allergic patients at the end of OIT, and Th1 cytokines of IFN-γ or TNF-α were not detected. Another study indicated that there were no significant changes in the cytokine profiles of IL-4, IL-5, IL-6, IL-10, or IL-12p70 during the cow's milk OIT process (Salmivesi et al., 2018). The possible reason is either that blood cytokine levels cannot completely reflect mucosal production or that mucosal and periphery Tregs have different functions. At present, cytokine detection serves as an available and relatively economic indicator for monitoring OIT effectiveness. In the future, more research should be conducted to explore the precise role and pattern of variation of these cytokines during OIT.

5.3 Th2 cells

As mentioned above, several studies have investigated the variation of cytokines secreted from Th2 cells during OIT. However, few studies have focused on the changes in Th2 cells themselves. Th2 cells are also important as mediators in initiating and orchestrating the allergic response, as well as in influencing

the course of AIT. At present, a major factor limiting the extensive use of such atopic disease-causing T cells as both therapeutic targets and clinical biomarkers is the lack of a suitable strategy to identify and distinguish these "bad" cells that involve allergic reactions from overall nonpathogenic Th2 cell types.

Recently, Wambre et al. (2012, 2017) have described a subset of human memory Th2 cells confined to atopic subjects that includes all allergen-specific Th2 cells. This proinflammatory Th2 cell subpopulation (named the Th2a cell subset) comprises terminally differentiated $CD4^+$ T cells characterized by stable co-expression of CRTh2, CD161, and CD49d, and low expression of CD45RB and CD27 ($CD4^+$, $CRTh2^+$, $CD161^+$, $CD49d^+$, $CD27^-$, and $CD45RB^-$). Th2a cells also exhibit numerous functional attributes distinct from those of conventional Th2 cells. Importantly, Th2a cells are specifically involved in the allergic response, but are virtually absent in non-allergic individuals. Moreover, food/component-specific Th2a cells are preferentially deleted after successful OIT, indicating that allergen-specific Th2a may represent a suitable therapeutic target and surrogate marker of clinical efficacy during AIT. In future, further detailed studies focusing on the Th2a subset may be of critical importance for understanding the mechanisms of action associated with the allergic process and successful AIT. They also may contribute greatly to response-monitoring and the design of appropriate immunomodulatory strategies (Wambre et al., 2017; Luce et al., 2020, 2021).

IL-4 is the quintessential Th2 cytokine produced by $CD4^+$ T cells (e.g., T follicular helper cells) in response to sensitization. As the important linker between Th2 cells and B cells, it can promote Th2 differentiation and the IgG/IgE isotype-switched antibody response. A combination of assessment of Th2a cells and residual IL-4-producing T cells may be clinically useful for monitoring OIT response and predicting SU durability.

5.4 DCs

DCs play a central role in initiating immune responses to culprit allergens and subsequently driving T cell differentiation (Ruiter and Shreffler, 2012; Liu et al., 2021). DCs, along with monocyte-macrophages, dictate oral tolerance against allergy by shaping the T-cell and subsequent B-cell antibody response. DCs from children with FA appear to generate more pro-inflammatory cytokines, and the $CD4^+$ T cells of subjects can be spontaneously activated to produce Th2 cytokines in the presence of FcεRI-bearing DCs (Frischmeyer-Guerrerio et al., 2011). In the context of oral tolerance, after innocuous food ingestion, goblet cells and intestinal resident macrophages ($CX3CR1^+$ macrophages) sample luminal food antigens and deliver them to LP $CD103^+$ $CX3CR1^-$ DCs (Bogunovic et al., 2009; Varol et al., 2009; Niess and Adler, 2010; McDole et al., 2012; Welty et al., 2013; Rivas et al., 2015; Esterhazy et al., 2016; Shiokawa et al., 2017; Kulkarni et al., 2020). After being activated by the antigen, these cDCs (also called migratory DCs) migrate via afferent lymphatics to the draining lymph nodes in a chemokine (C-C motif) receptor 7 (CCR7)-dependent manner. They then foster naive $CD4^+$ T to differentiate into peripheral Treg (pTreg) cells through epithelial cell-derived TGF-β and retinoic acid. Induction of Tregs can inhibit Th2-dependent allergic inflammation and MC degranulation (Fig. 1).

In general, the subset of cDCs (namely $CX3CR1^+$ DCs) are regarded as inflammatory, nonmigratory gut-resident T cells that have the capacity to promote production of TNF-α, induce Th17 cells, and activate Th1 cells. However, a recent study has suggested that $CX3CR1^+$ DCs may contribute to pTreg expansion and oral tolerance (Esterházy et al., 2016). In a murine oral tolerance model for FA, administration of a large amount of food additives significantly inhibited the migration of $CD103^+$ DCs into MLN and decreased the cell populations, thereby preventing the acquisition of oral tolerance (Yamashita et al., 2017). In the context of FA treatment, it was also shown that oral administration of probiotics could alleviate allergy symptoms and lead to an increase in Tregs induced by mucosal $CD103^+$ DCs (Fu et al., 2017). OIT seems to have an impact on circulating DCs, since the peripheral blood DCs after peanut OIT suppress methylation of FOXP3 CpG sites in effector T cells (Syed et al., 2014). Besides, a pilot study had demonstrated for the first time in humans that both OIT and SLIT are associated with changes in the innate functions of DCs, which may inhibit allergic reaction and promote tolerance development (Frischmeyer-Guerrerio et al., 2014). The therapeutic efficacy may be associated with the reduced pro-inflammatory cytokine (IL-6) secretion and increased IL-10 production by myeloid

DCs (mDCs). Moreover, increased IFN-α secretion from plasmacytoid DCs (pDCs) was also observed in subjects undergoing OIT. As IFN-α can act on mDCs to augment autocrine secretion of IL-10, which contributes to suppression of the FcεRI-dependent allergic response, OIT seems to exert pro-tolerogenic changes in both pDC and mDC innate immunity.

Notably, data focusing on the role of DCs in relation to FA and oral tolerance are almost entirely derived from mouse models and immunophenotyping of circulating cells with no distinct DC subsets. Small sample sizes in clinical trials also render it impossible to make more definitive statements regarding the relationships between the obtained DC findings and clinical outcomes. So far, no biomarker has been identified that is applicable for clinical use in OIT. Nevertheless, a greater understanding of how DCs contribute to the effectiveness of AIT and influence adaptive immune responses may inform the development of novel therapeutic strategies, both in FA and autoimmunity and transplantation. The present challenges provide the impetus to examine the above questions in larger clinical trials.

6 Potential cell markers

6.1 Myeloid-derived suppressor cells (candidate contributors to oral tolerance)

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells consisting of immature myeloid cells and myeloid progenitor cells. Besides playing an essential role in metastasis (Wang et al., 2019), such cells have the ability to suppress effector T-cell responses and activate Tregs by generating suppressive factors, including IL-10, TGF-β, and nitric oxide. Generally, MDSCs in mice are broadly identified on the basis of their coexpression of Gr1 and CD11b markers (Ji et al., 2016). The MDSCs can be further subdivided into two subsets: (1) polymorphonuclear MDSCs (PMN-MDSCs, $CD11b^{+}Ly6G^{+}Ly6C^{int}$), and (2) monocytic MDSCs (Mo-MDSCs, $CD11b^{+}Ly6G^{-}Ly6C^{hi}$). In the mouse models of OVA-induced FA and OIT, OIT induced systemic expansion of IL-10-producing $CD4^{+}$ T cells and MDSCs, particularly the monocytic MDSC subpopulation (Yoneyama et al., 2021). Notch signaling was involved in these processes. Moreover, the contribution of MDSCs to OIT-induced SU was confirmed by the prevention of establishment of SU after MDSC depletion with the anti-Gr1 antibody. Interestingly, repeated challenges with OVA reversely induced the development of severe allergic reactions in OVA-sensitized mice. Chronic and low-intensity stimulation or inflammation by OIT may contribute to the expansion of these suppressor cells, whereas excessive inflammation induced by the allergic process results in the activation/expansion of inflammatory cells, such as basophil and MC degranulation. A similar situation has also been observed in the immunological transition of MCs during FA and OIT processing. The frequencies of MDSCs and IL-10-producing CD4 T cells seem to be indicators of whether OIT can be successfully completed. For the moment, MDSCs may be regarded only as an indicator that reflects the immune status of subjects receiving immunotherapy. The practical application of MDSCs as a biomarker for predicting OIT outcomes remains to be confirmed with more supportive data.

6.2 Innate lymphoid cells (novel player in immune tolerance)

ILCs are new, intriguing lymphocytic populations that lack diversified antigen receptors expressed on T cells and B cells. They are largely tissue-resident cells and are involved with tissue development, remodeling, and inflammation (Vivier et al., 2018). ILCs can be categorized into subtypes including: (1) group 1 ILCs (ILC1s) and natural killer (NK) cells, (2) group 2 ILCs (ILC2s), and (3) group 3 ILCs (ILC3s) and lymphoid tissue inducer (LTi) cells, with comparable cytokine profiles to Th1, Th2, and Th17 subsets, respectively. ILC2s are heavily involved in allergic inflammation by amplifying the Th2 response. Also, they bear a resemblance to Th2 cells by sharing functional similarities. Usually, ILC2s reside in the skin, airway, and intestinal mucosa, and can respond to non-specific stimuli (such as IL-25, IL-33, and TSLP) after allergen exposure. Such cells appear to play a central role in allergic inflammation. Following resolution of inflammation, ILC2s may contribute to the re-establishment of epithelial barrier function and maintenance of tissue homeostasis through expression of the growth factor amphiregulin (Monticelli et al., 2015). Increasing evidence indicates that successful AIT is capable of leading to a significant reduction of

ILC2s in the peripheral blood of subjects (Lao-Araya et al., 2014; Fan et al., 2016; Mitthamsiri et al., 2018), suggesting that ILC2 detection may be an effective indicator for SCIT monitoring. Qualitative and quantitative measurements of ILC2s might also serve as promising biomarkers during FA-OIT processing. The precise impacts of these cells in the course of OIT and tolerance induction should further be investigated.

Human ILC3s are located mainly in the intestinal LP, and also contribute to the maintenance of mucosal immune tolerance. A recent study has suggested that activated $CD40L^+$ ILC3s may provide innate B cell help and are involved in an innate immunoregulatory mechanism through induction of IL-10-secreting immature transitional Breg (itBreg) differentiation (Komlósi et al., 2018). A decreased ILC3 level contributes to insufficient Breg-mediated immune tolerance in patients with various allergic disorders. Moreover, ILC3 also supports the generation, maintenance, and function of Tregs by controlling the production of IL-2 in the small intestine. ILC3s seem to exert a crucial role in the regulation of immunologic homeostasis and oral tolerance to dietary antigens (Zhou et al., 2019). Recently, a novel subset of ILCs with IL-10 production was identified as regulatory ILCs (ILCregs) (Wang et al., 2017). ILCregs exist in the gut and harbor a unique gene identity that is distinct from other subsets of ILCs and Tregs. They exert their regulatory functions by inhibiting inflammation via IL-10 and TGF-β1 production. All these ILC subsets appear to be important in orchestrating inflammation, immune tolerance, and tissue homeostasis, rendering them great potential indicators for FA-OIT assessment and SU predication. Uncovering the signaling circuits that manipulate the development and function of ILCs will help provide an integrated and comprehensive understanding of the regulation of tissue-specific immunity.

7 Omics-based tests

Emerging high-throughput “omics” technologies which provide molecular profiles for bio-specimens have been extensively used in experimental and clinical studies to reveal molecular subtypes and variation tendencies, elucidate the physico-pathological mechanisms of disease, develop mathematical models to predict clinical endpoints, and facilitate application of precision medicine (Mcshane et al., 2013; Yee et al., 2018; Giudice and Petsalaki, 2019; Crestani et al., 2020; Do et al., 2020; Mersha et al., 2021) (Table 2). In short, omics refers to measurable differences or changes in biological molecules, such as genes, epigenomes, proteins, metabolites, microflora, RNA, and environmental factors (Baar et al., 2014; Azad et al., 2015; di Girolamo et al., 2015; Greenhawt, 2015; Hesselmar et al., 2015; Li et al., 2015; Perkin et al., 2016; Turcanu et al., 2017; Kiyotani et al., 2018; Krutz et al., 2019; Mondoulet et al., 2019; Alhamwe et al., 2020; Kostara et al., 2020; Spertini, 2020) (Table 2). In a multi-center intervention trial comparing the efficacy of baked egg or egg OIT for the treatment of egg allergy (CoFAR7, NCT01846208) (Kosoy et al., 2016), measurement of transcriptional profiling by microarray was conducted to analyze PBMCs obtained from children with egg allergy, children with egg tolerance (to baked egg), and non-allergic children (controls). In combination with multiplex cytokine detection, the data revealed that increased expression of genes associated with allergic inflammation (carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) and cytokine-inducible Src homology 2 (SH2)-containing protein (CISH)) was linked with increased secretion of the cytokines IL-5, IL-9, and TNF-α. Transcriptional profiling is considered a useful tool to uncover novel immunologic processes in allergy and tolerance induction. In a pilot study (Crestani et al., 2020), mass spectrometry-based untargeted metabolomic profiling was performed to identify unique metabolites (candidate biomarkers) in sera collected from children with FA, asthma, or both. Lower levels of sphingolipids and ceramides were observed in children with FA, indicating the interplay between altered microbiota and immune cell subsets in the gut. In a mouse model of peanut allergy, elevated levels of uric acid (UA) were detected in mice undergoing sensitization by liquid chromatography-mass spectrometry (LC-MS)-based metabolomics analysis (Kong et al., 2015). During sensitization, depletion of UA prevented the development of peanut-specific IgE and IgG1, as well as anaphylaxis, while exogenous delivery of UA crystals (monosodium urate) restored the allergic phenotype.

Gut microbes can shape the development of the host immune system, and in turn the host immune status can influence the composition of the microbiome.

Table 2 Overview of current omics technologies

Omics	Scope of application	Samples	Methods	Discoveries
Genomics	Gene/exome sequencing, SNPs, mutations	DNAs	DNA sequencing	Genetic associations/inherited phenotypes with FA; discovery of genes involved in immune cell function
Epigenomics	Histone modification, CpG methylations	DNAs	Methylation array; genome-wide DNA methylation analysis; RNA methylation; ChIP-seq; ATAC-seq	Potential FA/AIT response biomarkers
Transcriptomics	Transcriptional regulation, sequencing	RNAs	Low-input RNA library sequencing; targeted RNA sequencing	Gene expression in immune cells is associated with FA and tolerant subjects; further characterization of allergen proteins
Proteomics	Peptide/protein identification, allergens, post-translational modification	Proteins	NMR; GC-MS; LC-MS; LC-QTOF-MS	Contributing to the diagnosis/prognosis of FA; characterization and quality testing of allergen proteins or immunotherapy products
Metabolomics	Signaling molecules, hormones, metabolites	Proteins	Similar to proteomics	Identifying metabolic profiles/pathway activities from host system or microbiome-derived products which are associated with FA
Microbiomics	Microflora, probiotics, dietary supplements	DNAs, RNAs, proteins, environments	MS, 16S rRNA sequencing	Potential biomarkers for FA development or resolution; potential probiotic candidates for OIT therapy
Exposomics	Pathogens, environmental exposures, drugs, toxins	Environments	MS	Risk factors contributing to FA incidence/prevalence/severity; offering protection against FA epidemic (e.g., early allergic food introduction)

SNPs, single nucleotide polymorphisms; FA, food allergy; CpG, cytosine-phosphate-guanosine; ChIP-seq, chromatin immunoprecipitation-based sequencing; ATAC-seq, assay for transposase-accessible chromatin with high-throughput sequencing; AIT, allergen-specific immunotherapy; NMR, nuclear magnetic resonance; GC-MS, gas chromatography-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; LC-QTOF-MS, liquid chromatography quadrupole time-of-flight mass spectrometry; rRNA, ribosomal RNA; OIT, oral immunotherapy.

Manipulating the intestinal flora has been proposed as an adjunct to AIT for improving efficacy and safety (Rachid and Chatila, 2016; Aitoro et al., 2017; Canani et al., 2019). Bunyavanich et al. (2016) published the first study to define the relationship between the microbiome and FA resolution, based on 226 children with cow's milk allergy. By using high-throughput sequencing and microbiological analysis, enriched taxa from the Clostridia class and Firmicutes phylum in the infant flora (3–6 months) were observed in subjects with milk resolution by eight years of age. Previous studies suggested that colonization of mice by a mix of *Clostridium* strains provided an environment rich in TGF-β, and affected the number and function $Foxp3^+$ Tregs in the colon (Atarashi et al., 2011, 2013). As described above, $Foxp3^+$ Tregs play a critical role in oral tolerance. In a recent study (He et al., 2021), alpha diversity of the gut microbiota increased significantly in most allergic participants after a long-term (52-week) peanut OIT. Therefore, not only specific taxa of microflora can be studied as probiotic candidates for FA treatment, but also the changes of microflora during OIT can serve as biomarkers for efficacy prediction. Moreover, metabolites, such as short-chain fatty acids, are crucial metabolic products of gut microbiota responsible for protective effects against FA. These compounds are also involved in epigenetic regulation of the immune system. Considering the complicated mechanisms and comprehensive components related to allergy and immunotolerance, further exploration of omics analysis technology (e.g., single-cell omics approaches), as well as optimized combinations of testing indexes (e.g., combining genomics, epigenomics, and transcriptomics for functional characterization, and combining microbiomics, metaboiomics, and proteomics for integrative analysis) may lead to the identification of new promising biomarkers and potential therapeutic targets (Table 2). In the next

decades, parallel advances in bioinformatics and computational techniques will better enable the integration, analysis, and interpretation of these exponentially growing datasets and eventually provide the possibility of personalized or/and precision medicine for FA and AIT.

8 Conclusions

Typically, OIT exerts therapeutic effects by affecting many components of the host immune system. OIT-induced immune changes primarily include an early decrease in MC and basophil activation, upregulated generation of allergen-specific Tregs and Bregs with decreased Th2 production, and changes in allergen-specific antibodies usually characterized by a temporary increase followed by a decrease in sIgE and a gradual increase in sIgG4. Nevertheless, the precise mechanisms underlying these changes and the precise sequence of immune events in the re-establishment of oral tolerance are still poorly understood. Ideal biomarkers with the capacity to accurately predict desensitization and/or tolerance development are needed to implement OIT. At least for safety, this is essential to inform allergic subjects about treatment duration and risk behavior if AIT is conducted. Several indicators such as DCs, B cell, macrophages, regulatory MCs, and ILC subsets have not been well studied in the context of predicting AIT outcomes. Currently available biomarkers in clinics (such as SPTs, BATs, cytokines, specific antibody levels and ratios, and Treg detection) appear to be insufficient to reliably distinguish subjects who have achieved desensitization or SU, and inadequately reflect the subtle immune status and regulatory processes occurring during FA and AIT. Continuing efforts are needed to greatly expand the repertoire of available biomarkers and to discover novel biomarkers for providing personalized and precise approaches to OIT.

Acknowledgments

This work was supported by the Talent Training Project of Northwest Women's and Children's Hospital (No. 2018LQ01), the National Natural Science Foundation of China (No. 82070858), and the Natural Science Foundation of Shaanxi Province (Nos. 2020JQ-941 and 2021JQ-911), China. We thank Dr. Ning JING (Xi'an Sanmin Community Health Service Station) for language polishing of the original version of this manuscript, and Jingwen ZHU (Xi'an Gaoxin No. 1 Primary School) for color design of Fig. 1. In addition, Dr. Haitao ZHU wants to thank, in particular, the patience, generous heart and love from Dr. Ning JING over the past 12 years. Haitao ZHU (tarn1000) will always cherish you.

Author contributions

Haitao ZHU conceived the central idea and completed the manuscript. Kaifa TANG and Guoqiang CHEN contributed to manuscript review. Zhongwei LIU finalized the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Haitao ZHU, Kaifa TANG, Guoqiang CHEN, and Zhongwei LIU declare that they have no conflict of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

References

Aitoro R, Paparo L, Amoroso A, et al., 2017. Gut microbiota as a target for preventive and therapeutic intervention against food allergy. *Nutrients*, 9(7):672. https://doi.org/10.3390/nu9070672

Akdis M, Akdis CA, 2014. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol*, 133(3):621-631. https://doi.org/10.1016/j.jaci.2013.12.1088

Akdis M, Aab A, Altunbulakli C, et al., 2016. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: receptors, functions, and roles in diseases. *J Allergy Clin Immunol*, 138(4):984-1010. https://doi.org/10.1016/j.jaci.2016.06.033

Alhamwe BA, Meulenbroek LAPM, Veening-Griffioen DH, et al., 2020. Decreased histone acetylation levels at Th1 and regulatory loci after induction of food allergy. *Nutrients*, 12(10):3193. https://doi.org/10.3390/nu12103193

Altin JA, Goodnow CC, Cook MC, 2012. IL-10^{+}CTLA-4^{+} Th2 inhibitory cells form in a Foxp3-independent, IL-2-dependent manner from Th2 effectors during chronic inflammation. *J Immunol*, 188(11):5478-5488. https://doi.org/10.4049/jimmunol.1102994

Amano T, Furuno T, Hirashima N, et al., 2001. Dynamics of intracellular granules with CD63-GFP in rat basophilic leukemia cells. *J Biochem*, 129(5):739-744. https://doi.org/10.1093/oxfordjournals.jbchem.a002914

Anagnostou K, 2018. Anaphylaxis in children: epidemiology, risk factors and management. *Curr Pediatr Rev*, 14(3): 180-186. https://doi.org/10.2174/1573396314666180507115115

Anagnostou K, Islam S, King Y, et al., 2014. Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial. *Lancet*, 383(9925):1297-1304. https://doi.org/10.1016/S0140-6736(13)62301-6

Ang WXG, Church AM, Kulis M, et al., 2016. Mast cell desensitization inhibits calcium flux and aberrantly remodels actin. *J Clin Invest*, 126(11):4103-4118. https://doi.org/10.1172/JCI87492

Ansotegui IJ, Melioli G, Canonica GW, et al., 2020. IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper. *World Allergy Organ J*, 13(2):100080. https://doi.org/10.1016/j.waojou.2019.100080

Atarashi K, Tanoue T, Shima T, et al., 2011. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science*, 331(6015):337-341. https://doi.org/10.1126/science.1198469

Atarashi K, Tanoue T, Oshima K, et al., 2013. T_{reg} induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*, 500(7461):232-236. https://doi.org/10.1038/nature12331

Attias M, Al-Aubodah T, Piccirillo CA, 2019. Mechanisms of human FoxP3$^+$ T_{reg} cell development and function in health and disease. *Clin Exp Immunol*, 197(1):36-51. https://doi.org/10.1111/cei.13290

Azad MB, Konya T, Guttman DS, et al., 2015. Infant gut microbiota and food sensitization: associations in the first year of life. *Clin Exp Allergy*, 45(3):632-643. https://doi.org/10.1111/cea.12487

Baar A, Pahr S, Constantin C, et al., 2014. The high molecular weight glutenin subunit Bx7 allergen from wheat contains repetitive IgE epitopes. *Allergy*, 69(10):1316-1323. https://doi.org/10.1111/all.12464

Bahri R, Custovic A, Korosec P, et al., 2018. Mast cell activation test in the diagnosis of allergic disease and anaphylaxis. *J Allergy Clin Immunol*, 142(2):485-496.e16. https://doi.org/10.1016/j.jaci.2018.01.043

Berger A, 2000. Th1 and Th2 responses: what are they? *BMJ*, 321(7258):424. https://doi.org/10.1136/bmj.321.7258.424

Beyer K, Ellman-Grunther L, Jarvinen KM, et al., 2003. Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. *J Allergy Clin Immunol*, 112(1):202-207. https://doi.org/10.1067/mai.2003.1621

Bock SA, Sampson HA, Atkins FM, et al., 1988. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol*, 82(6):986-997. https://doi.org/10.1016/0091-6749(88)90135-2

Bock SA, Muñoz-Furlong A, Sampson HA, 2007. Further fatalities caused by anaphylactic reactions to food, 2001–2006. *J Allergy Clin Immunol*, 119(4):1016-1018. https://doi.org/10.1016/j.jaci.2006.12.622

Bogunovic M, Ginhoux F, Helft J, et al., 2009. Origin of the lamina propria dendritic cell network. *Immunity*, 31(3):513-525. https://doi.org/10.1016/j.immuni.2009.08.010

Boonpiyathad T, Meyer N, Moniuszko M, et al., 2017. High-dose bee venom exposure induces similar tolerogenic B-cell responses in allergic patients and healthy beekeepers. *Allergy*, 72(3):407-415. https://doi.org/10.1111/all.12966

Boonpiyathad T, van de Veen W, Wirz O, et al., 2019. Role of Der p 1-specific B cells in immune tolerance during 2 years of house dust mite-specific immunotherapy. *J Allergy Clin Immunol*, 143(3):1077-1086.e10. https://doi.org/10.1016/j.jaci.2018.10.061

Bunyavanich S, Shen N, Grishin A, et al., 2016. Early-life gut microbiome composition and milk allergy resolution. *J Allergy Clin Immunol*, 138(4):1122-1130. https://doi.org/10.1016/j.jaci.2016.03.041

Burks AW, Jones SM, Wood RA, et al., 2012. Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med*, 367(3):233-243. https://doi.org/10.1056/NEJMoa1200435

Burton OT, Rivas MN, Zhou JS, et al., 2014a. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. *Immunity*, 41(1):141-151. https://doi.org/10.1016/j.immuni.2014.05.017

Burton OT, Logsdon SL, Zhou JS, et al., 2014b. Oral immunotherapy induces IgG antibodies that act through FcγRIIb to suppress IgE-mediated hypersensitivity. *J Allergy Clin Immunol*, 134(6):1310-1317.e6. https://doi.org/10.1016/j.jaci.2014.05.042

Burton OT, Tamayo JM, Stranks AJ, et al., 2018. Allergen-specific IgG antibody signaling through FcγRIIb promotes food tolerance. *J Allergy Clin Immunol*, 141(1):189-201.e3. https://doi.org/10.1016/j.jaci.2017.03.045

Calderon TE, Ferrero M, Marino GM, et al., 2010. Meat-specific IgG and IgA antibodies coexist with IgE antibodies in sera from allergic patients: clinical association and modulation by exclusion diet. *J Biol Regul Homeost Agents*, 24(3):261-271.

Canani RB, Paparo L, Nocerino R, et al., 2019. Gut microbiome as target for innovative strategies against food allergy. *Front Immunol*, 10:191. https://doi.org/10.3389/fimmu.2019.00191

Carrier Y, Yuan J, Kuchroo VK, et al., 2007. Th3 cells in peripheral tolerance. I. Induction of Foxp3-positive regulatory T cells by Th3 cells derived from TGF-β T cell-transgenic mice. *J Immunol*, 178(1):179-185. https://doi.org/10.4049/jimmunol.178.1.179

Cassard L, Jönsson F, Arnaud S, et al., 2012. Fcγ receptors inhibit mouse and human basophil activation. *J Immunol*, 189(6):2995-3006. https://doi.org/10.4049/jimmunol.1200968

Chen CY, Lee JB, Liu B, et al., 2015. Induction of interleukin-9-producing mucosal mast cells promotes susceptibility to IgE-mediated experimental food allergy. *Immunity*, 43(4):788-802. https://doi.org/10.1016/j.immuni.2015.08.020

Chinthrajah RS, Hernandez JD, Boyd SD, et al., 2016. Molecular and cellular mechanisms of food allergy and food tolerance. *J Allergy Clin Immunol*, 137(4):984-997. https://doi.org/10.1016/j.jaci.2016.02.004

Chinthrajah RS, Purington N, Andorf S, et al., 2019. Sustained outcomes in oral immunotherapy for peanut allergy (POISED study): a large, randomised, double-blind,

placebo-controlled, phase 2 study. *Lancet*, 394(10207): 1437-1449.
https://doi.org/10.1016/S0140-6736(19)31793-3

Chinuki Y, Kaneko S, Dekio I, et al., 2012. CD203c expression-based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol*, 129(5):1404-1406.
https://doi.org/10.1016/j.jaci.2012.02.049

Commins SP, James HR, Stevens W, et al., 2014. Delayed clinical and *ex vivo* response to mammalian meat in patients with IgE to galactose-alpha-1,3-galactose. *J Allergy Clin Immunol*, 134(1):108-115.e11.
https://doi.org/10.1016/j.jaci.2014.01.024

Costantini VP, Nguyen K, Lyski Z, et al., 2022. Development and validation of an enzyme immunoassay for detection and quantification of SARS-CoV-2 salivary IgA and IgG. *J Immunol*, 208(6):1500-1508.
https://doi.org/10.4049/jimmunol.2100934

Crestani E, Harb H, Charbonnier LM, et al., 2020. Untargeted metabolomic profiling identifies disease-specific signatures in food allergy and asthma. *J Allergy Clin Immunol*, 145(3):897-906.
https://doi.org/10.1016/j.jaci.2019.10.014

Crivellato E, Travan L, Ribatti D, 2010. Mast cells and basophils: a potential link in promoting angiogenesis during allergic inflammation. *Int Arch Allergy Immunol*, 151(2): 89-97.
https://doi.org/10.1159/000235998

Cucu T, de Meulenaer B, Bridts C, et al., 2012. Impact of thermal processing and the Maillard reaction on the basophil activation of hazelnut allergic patients. *Food Chem Toxicol*, 50(5):1722-1728.
https://doi.org/10.1016/j.fct.2012.02.069

Cuomo B, Indirli GC, Bianchi A, et al., 2017. Specific IgE and skin prick tests to diagnose allergy to fresh and baked cow's milk according to age: a systematic review. *Ital J Pediatr*, 43:93.
https://doi.org/10.1186/s13052-017-0410-8

de Quiros EB, Seoane-Reula E, Alonso-Lebrero E, et al., 2018. The role of regulatory T cells in the acquisition of tolerance to food allergens in children. *Allergol Immunopathol (Madr)*, 46(6):612-618.
https://doi.org/10.1016/j.aller.2018.02.002

di Girolamo F, Muraca M, Mazzina O, et al., 2015. Proteomic applications in food allergy: food allergenomics. *Curr Opin Allergy Clin Immunol*, 15(3):259-266.
https://doi.org/10.1097/aci.0000000000000160

Do AN, Watson CT, Cohain AT, et al., 2020. Dual transcriptomic and epigenomic study of reaction severity in peanut-allergic children. *J Allergy Clin Immunol*, 145(4):1219-1230.
https://doi.org/10.1016/j.jaci.2019.10.040

Dreskin SC, Germinaro M, Reinhold D, et al., 2019. IgE binding to linear epitopes of Ara h 2 in peanut allergic preschool children undergoing oral immunotherapy. *Pediatr Allergy Immunol*, 30(8):817-823.
https://doi.org/10.1111/pai.13117

Dubois B, Chapat L, Goubier A, et al., 2003. Innate $CD4^+CD25^+$ regulatory T cells are required for oral tolerance and inhibition of $CD8^+$ T cells mediating skin inflammation. *Blood*, 102(9):3295-3301.
https://doi.org/10.1182/blood-2003-03-0727

Ebruster H, 1959. The prick test, a recent cutaneous test for the diagnosis of allergic disorders. *Wien Klin Wochenschr*, 71:551-554.

Eigenmann PA, Sampson HA, 1998. Interpreting skin prick tests in the evaluation of food allergy in children. *Pediatr Allergy Immunol*, 9(4):186-191.
https://doi.org/10.1111/j.1399-3038.1998.tb00371.x

Eiwegger T, Hung L, San Diego KE, et al., 2019. Recent developments and highlights in food allergy. *Allergy*, 74(12):2355-2367.
https://doi.org/10.1111/all.14082

Escudero C, del Río PR, Sánchez-García S, et al., 2015. Early sustained unresponsiveness after short-course egg oral immunotherapy: a randomized controlled study in egg-allergic children. *Clin Exp Allergy*, 45(12):1833-1843.
https://doi.org/10.1111/cea.12604

Esterházy D, Loschko J, London M, et al., 2016. Classical dendritic cells are required for dietary antigen-mediated induction of peripheral T_{reg} cells and tolerance. *Nat Immunol*, 17(5):545-555.
https://doi.org/10.1038/ni.3408

Fan DC, Wang XD, Wang CS, et al., 2016. Suppression of immunotherapy on group 2 innate lymphoid cells in allergic rhinitis. *Chin Med J (Engl)*, 129(23):2824-2828.
https://doi.org/10.4103/0366-6999.194642

Feng T, Elson CO, Cong YZ, 2011. Treg cell-IgA axis in maintenance of host immune homeostasis with microbiota. *Int Immunopharmacol*, 11(5):589-592.
https://doi.org/10.1016/j.intimp.2010.11.016

Flinterman AE, Knol EF, Lencer DA, et al., 2008. Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy. *J Allergy Clin Immunol*, 121(3):737-743.e10.
https://doi.org/10.1016/j.jaci.2007.11.039

Frati F, Incorvaia C, Cavaliere C, et al., 2018. The skin prick test. *J Biol Regul Homeost Agents*, 32(1 Suppl 1):19-24.

Frischmeyer-Guerrerio PA, Guerrerio AL, Chichester KL, et al., 2011. Dendritic cell and T cell responses in children with food allergy. *Clin Exp Allergy*, 41(1):61-71.
https://doi.org/10.1111/j.1365-2222.2010.03606.x

Frischmeyer-Guerrerio PA, Keet CA, Guerrerio AL, et al., 2014. Modulation of dendritic cell innate and adaptive immune functions by oral and sublingual immunotherapy. *Clin Immunol*, 155(1):47-59.
https://doi.org/10.1016/j.clim.2014.08.006

Frischmeyer-Guerrerio PA, Masilamani M, Gu WJ, et al., 2017. Mechanistic correlates of clinical responses to omalizumab in the setting of oral immunotherapy for milk allergy. *J Allergy Clin Immunol*, 140(4):1043-1053.e8.
https://doi.org/10.1016/j.jaci.2017.03.028

Fu LL, Song JY, Wang C, et al., 2017. *Bifidobacterium infantis* potentially alleviates shrimp tropomyosin-induced allergy by tolerogenic dendritic cell-dependent induction of regulatory T cells and alterations in gut microbiota. *Front*

Immunol, 8:1536. https://doi.org/10.3389/fimmu.2017.01536

Gernez Y, Nowak-Wegrzyn A, 2017. Immunotherapy for food allergy: are we there yet? *J Allergy Clin Immunol Pract*, 5(2):250-272. https://doi.org/10.1016/j.jaip.2016.12.004

Giudice G, Petsalaki E, 2019. Proteomics and phosphoproteomics in precision medicine: applications and challenges. *Brief Bioinform*, 20(3):767-777. https://doi.org/10.1093/bib/bbx141

Glaumann S, Nopp A, Johansson SGO, et al., 2012. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. *Allergy*, 67(2): 242-247. https://doi.org/10.1111/j.1398-9995.2011.02754.x

Gober LM, Eckman JA, Sterba PM, et al., 2007. Expression of activation markers on basophils in a controlled model of anaphylaxis. *J Allergy Clin Immunol*, 119(5):1181-1188. https://doi.org/10.1016/j.jaci.2007.01.008

Gorelik M, Narisety SD, Guerrerio AL, et al., 2015. Suppression of the immunologic response to peanut during immunotherapy is often transient. *J Allergy Clin Immunol*, 135(5):1283-1292. https://doi.org/10.1016/j.jaci.2014.11.010

Greenhawt M, 2015. The learning early about peanut allergy study: the benefits of early peanut introduction, and a new horizon in fighting the food allergy epidemic. *Pediatr Clin North Am*, 62(6):1509-1521. https://doi.org/10.1016/j.pcl.2015.07.010

Gupta RS, Lau CH, Hamilton RG, et al., 2014. Predicting outcomes of oral food challenges by using the allergen-specific IgE-total IgE ratio. *J Allergy Clin Immunol Pract*, 2(3):300-305. https://doi.org/10.1016/j.jaip.2013.12.006

Hadis U, Wahl B, Schulz O, et al., 2011. Intestinal tolerance requires gut homing and expansion of $FoxP3^+$ regulatory T cells in the lamina propria. *Immunity*, 34(2):237-246. https://doi.org/10.1016/j.immuni.2011.01.016

Hao GD, Zheng YW, Wang ZX, et al., 2016. High correlation of specific IgE sensitization between birch pollen, soy and apple allergens indicates pollen-food allergy syndrome among birch pollen allergic patients in northern China. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 17(5):399-404. https://doi.org/10.1631/jzus.B1500279

He ZY, Vadali VLG, Szabady RL, et al., 2021. Increased diversity of gut microbiota during active oral immunotherapy in peanut-allergic adults. *Allergy*, 76(3):927-930. https://doi.org/10.1111/all.14540

Heeringa JJ, McKenzie CI, Varese N, et al., 2020. Induction of IgG_2 and IgG_4 B-cell memory following sublingual immunotherapy for ryegrass pollen allergy. *Allergy*, 75(5): 1121-1132. https://doi.org/10.1111/all.14073

Hesselmar B, Hicke-Roberts A, Wennergren G, 2015. Allergy in children in hand versus machine dishwashing. *Pediatrics*, 135(3):e590-e597. https://doi.org/10.1542/peds.2014-2968

Hoh RA, Joshi SA, Liu Y, et al., 2016. Single B-cell deconvolution of peanut-specific antibody responses in allergic patients. *J Allergy Clin Immunol*, 137(1):157-167. https://doi.org/10.1016/j.jaci.2015.05.029

Hong HY, Liao SM, Chen FH, et al., 2020. Role of IL-25, IL-33, and TSLP in triggering united airway diseases toward type 2 inflammation. *Allergy*, 75(11):2794-2804. https://doi.org/10.1111/all.14526

Hourihane JOB, Beyer K, Abbas A, et al., 2020. Efficacy and safety of oral immunotherapy with AR101 in European children with a peanut allergy (ARTEMIS): a multicentre, double-blind, randomised, placebo-controlled phase 3 trial. *Lancet Child Adolesc Health*, 4(10):728-739. https://doi.org/10.1016/S2352-4642(20)30234-0

Imakiire R, Fujisawa T, Nagao M, et al., 2020. Basophil activation test based on CD203c expression in the diagnosis of fish allergy. *Allergy Asthma Immunol Res*, 12(4):641-652. https://doi.org/10.4168/aair.2020.12.4.641

Janzi M, Kull I, Sjöberg R, et al., 2009. Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. *Clin Immunol*, 133(1):78-85. https://doi.org/10.1016/j.clim.2009.05.014

Järvinen KM, Sicherer SH, 2012. Diagnostic oral food challenges: procedures and biomarkers. *J Immunol Methods*, 383(1-2):30-38. https://doi.org/10.1016/j.jim.2012.02.019

Ji JJ, Xu JJ, Zhao SL, et al., 2016. Myeloid-derived suppressor cells contribute to systemic lupus erythaematosus by regulating differentiation of Th17 cells and Tregs. *Clin Sci (Lond)*, 130(16):1453-1467. https://doi.org/10.1042/cs20160311

Jones SM, Pons L, Roberts JL, et al., 2009. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol*, 124(2):292-300.e97. https://doi.org/10.1016/j.jaci.2009.05.022

Jones SM, Burks AW, Keet C, et al., 2016. Long-term treatment with egg oral immunotherapy enhances sustained unresponsiveness that persists after cessation of therapy. *J Allergy Clin Immunol*, 137(4):1117-1127.e10. https://doi.org/10.1016/j.jaci.2015.12.1316

Jutel M, Akdis M, Budak F, et al., 2003. IL-10 and TGF-β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol*, 33(5):1205-1214. https://doi.org/10.1002/eji.200322919

Kim EH, Perry TT, Wood RA, et al., 2020. Induction of sustained unresponsiveness after egg oral immunotherapy compared to baked egg therapy in children with egg allergy. *J Allergy Clin Immunol*, 146(4):851-862.e10. https://doi.org/10.1016/j.jaci.2020.05.040

Kim YH, Kim YS, Park Y, et al., 2020. Investigation of basophil activation test for diagnosing milk and egg allergy in younger children. *J Clin Med*, 9(12):3942. https://doi.org/10.3390/jcm9123942

Kiyotani K, Mai TH, Yamaguchi R, et al., 2018. Characterization of the B-cell receptor repertoires in peanut allergic subjects undergoing oral immunotherapy. *J Hum Genet*,

63(2):239-248.
https://doi.org/10.1038/s10038-017-0364-0

Komata T, Söderström L, Borres MP, et al., 2007. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. *J Allergy Clin Immunol*, 119(5):1272-1274.
https://doi.org/10.1016/j.jaci.2007.01.038

Komlósi ZI, Kovács N, van de Veen W, et al., 2018. Human CD40 ligand-expressing type 3 innate lymphoid cells induce IL-10-producing immature transitional regulatory B cells. *J Allergy Clin Immunol*, 142(1):178-194.e11.
https://doi.org/10.1016/j.jaci.2017.07.046

Kong J, Chalcraft K, Mandur TS, et al., 2015. Comprehensive metabolomics identifies the alarmin uric acid as a critical signal for the induction of peanut allergy. *Allergy*, 70(5):495-505.
https://doi.org/10.1111/all.12579

Kosoy R, Agashe C, Grishin A, et al., 2016. Transcriptional profiling of egg allergy and relationship to disease phenotype. *PLoS ONE*, 11(10):e0163831.
https://doi.org/10.1371/journal.pone.0163831

Kostara M, Chondrou V, Sgourou A, et al., 2020. HLA polymorphisms and food allergy predisposition. *J Pediatr Genet*, 9(2):77-86.
https://doi.org/10.1055/s-0040-1708521

Krishna MT, Huissoon AP, 2011. Clinical immunology review series: an approach to desensitization. *Clin Exp Immunol*, 163(2):131-146.
https://doi.org/10.1111/j.1365-2249.2010.04296.x

Krutz NL, Winget J, Ryan CA, et al., 2019. Proteomic and bioinformatic analyses for the identification of proteins with low allergenic potential for hazard assessment. *Toxicol Sci*, 170(1):210-222.
https://doi.org/10.1093/toxsci/kfz078

Kubo M, 2018. Mast cells and basophils in allergic inflammation. *Curr Opin Immunol*, 54:74-79.
https://doi.org/10.1016/j.coi.2018.06.006

Kulis M, Saba K, Kim EH, et al., 2012. Increased peanut-specific IgA levels in saliva correlate with food challenge outcomes after peanut sublingual immunotherapy. *J Allergy Clin Immunol*, 129(4):1159-1162.
https://doi.org/10.1016/j.jaci.2011.11.045

Kulis M, Yue XH, Guo RS, et al., 2019. High- and low-dose oral immunotherapy similarly suppress pro-allergic cytokines and basophil activation in young children. *Clin Exp Allergy*, 49(2):180-189.
https://doi.org/10.1111/cea.13256

Kulkarni DH, Gustafsson JK, Knoop KA, et al., 2020. Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. *Mucosal Immunol*, 13(2):271-282.
https://doi.org/10.1038/s41385-019-0240-7

Kurashima Y, Kunisawa J, Higuchi M, et al., 2007. Sphingosine 1-phosphate-mediated trafficking of pathogenic Th2 and mast cells for the control of food allergy. *J Immunol*, 179(3):1577-1585.
https://doi.org/10.4049/jimmunol.179.3.1577

Lao-Araya M, Steveling E, Scadding GW, et al., 2014. Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy. *J Allergy Clin Immunol*, 134(5):1193-1195.e4.
https://doi.org/10.1016/j.jaci.2014.07.029

Leung DYM, Sampson HA, Yunginger JW, et al., 2003. Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med*, 348(11):986-993.
https://doi.org/10.1056/NEJMoa022613

Leveson-Gower DB, Sega EI, Kalesnikoff J, et al., 2013. Mast cells suppress murine GVHD in a mechanism independent of $CD4^+CD25^+$ regulatory T cells. *Blood*, 122(22):3659-3665.
https://doi.org/10.1182/blood-2013-08-519157

Li J, Fung I, Glessner JT, et al., 2015. Copy number variations in *CTNNA3* and *RBFOX1* associate with pediatric food allergy. *J Immunol*, 195(4):1599-1607.
https://doi.org/10.4049/jimmunol.1402310

Liu EG, Yin XY, Swaminathan A, et al., 2021. Antigen-presenting cells in food tolerance and allergy. *Front Immunol*, 11:616020.
https://doi.org/10.3389/fimmu.2020.616020

Lopes JP, Sicherer S, 2020. Food allergy: epidemiology, pathogenesis, diagnosis, prevention, and treatment. *Curr Opin Immunol*, 66:57-64.
https://doi.org/10.1016/j.coi.2020.03.014

Luce S, Chinthrajah S, Lyu SC, et al., 2020. Th2A and Th17 cell frequencies and regulatory markers as follow-up biomarker candidates for successful multifood oral immunotherapy. *Allergy*, 75(6):1513-1516.
https://doi.org/10.1111/all.14180

Luce S, Batard T, Floch VBL, et al., 2021. Decrease in $CD38^+$ TH2A cell frequencies following immunotherapy with house dust mite tablet correlates with humoral responses. *Clin Exp Allergy*, 51(8):1057-1068.
https://doi.org/10.1111/cea.13891

Lúðvíksson BR, Arason GJ, Thorarensen O, et al., 2005. Allergic diseases and asthma in relation to serum immunoglobulins and salivary immunoglobulin A in pre-school children: a follow-up community-based study. *Clin Exp Allergy*, 35(1):64-69.
https://doi.org/10.1111/j.1365-2222.2005.02141.x

Marrs T, Flohr C, Perkin MR, 2015. Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial: a critical appraisal. *Br J Dermatol*, 173(5):1125-1129.
https://doi.org/10.1111/bjd.14161

Martínez-Botas J, Rodríguez-Álvarez M, Cerecedo I, et al., 2015. Identification of novel peptide biomarkers to predict safety and efficacy of cow's milk oral immunotherapy by peptide microarray. *Clin Exp Allergy*, 45(6):1071-1084.
https://doi.org/10.1111/cea.12528

McDole JR, Wheeler LW, McDonald KG, et al., 2012. Goblet cells deliver luminal antigen to $CD103^+$ dendritic cells in the small intestine. *Nature*, 483(7389):345-349.
https://doi.org/10.1038/nature10863

McShane LM, Cavenagh MM, Lively TG, et al., 2013. Criteria for the use of omics-based predictors in clinical trials:

explanation and elaboration. *BMC Med*, 11:220. https://doi.org/10.1186/1741-7015-11-220

Méndez-Enríquez E, Salomonsson M, Eriksson J, et al., 2022. IgE cross-linking induces activation of human and mouse mast cell progenitors. *J Allergy Clin Immunol*, 149(4): 1458-1463. https://doi.org/10.1016/j.jaci.2021.08.019

Mersha TB, Afanador Y, Johansson E, et al., 2021. Resolving clinical phenotypes into endotypes in allergy: molecular and omics approaches. *Clin Rev Allergy Immunol*, 60(2): 200-219. https://doi.org/10.1007/s12016-020-08787-5

Mitthamsiri W, Pradubpongsa P, Sangasapaviliya A, et al., 2018. Decreased CRTH2 expression and response to allergen re-stimulation on innate lymphoid cells in patients with allergen-specific immunotherapy. *Allergy Asthma Immunol Res*, 10(6):662-674. https://doi.org/10.4168/aair.2018.10.6.662

Mondoulet L, Dioszeghy V, Busato F, et al., 2019. *Gata3* hypermethylation and *Foxp3* hypomethylation are associated with sustained protection and bystander effect following epicutaneous immunotherapy in peanut-sensitized mice. *Allergy*, 74(1):152-164. https://doi.org/10.1111/all.13479

Monticelli LA, Osborne LC, Noti M, et al., 2015. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci USA*, 112(34):10762-10767. https://doi.org/10.1073/pnas.1509070112

Moraly T, de Chambure DP, Verdun S, et al., 2020. Oral immunotherapy for hazelnut allergy: a single-center retrospective study on 100 patients. *J Allergy Clin Immunol Pract*, 8(2):704-709.e4. https://doi.org/10.1016/j.jaip.2019.10.045

Morita H, Arae K, Unno H, et al., 2015. An interleukin-33-mast cell-interleukin-2 axis suppresses papain-induced allergic inflammation by promoting regulatory T cell numbers. *Immunity*, 43(1):175-186. https://doi.org/10.1016/j.immuni.2015.06.021

Mousallem T, Burks AW, 2012. Immunology in the clinic review series; focus on allergies: immunotherapy for food allergy. *Clin Exp Immunol*, 167(1):26-31. https://doi.org/10.1111/j.1365-2249.2011.04499.x

Nachshon L, Goldberg MR, Katz Y, et al., 2018. Long-term outcome of peanut oral immunotherapy-real-life experience. *Pediatr Allergy Immunol*, 29(5):519-526. https://doi.org/10.1111/pai.12914

Nagakura KI, Yanagida N, Sato S, et al., 2018. Low-dose oral immunotherapy for children with anaphylactic peanut allergy in Japan. *Pediatr Allergy Immunol*, 29(5):512-518. https://doi.org/10.1111/pai.12898

Nagakura KI, Sato S, Miura Y, et al., 2021. A randomized trial of oral immunotherapy for pediatric cow's milk-induced anaphylaxis: heated vs unheated milk. *Pediatr Allergy Immunol*, 32(1):161-169. https://doi.org/10.1111/pai.13352

Nakajima S, Kabata H, Kabashima K, et al., 2020. Anti-TSLP antibodies: targeting a master regulator of type 2 immune responses. *Allergol Int*, 69(2):197-203. https://doi.org/10.1016/j.alit.2020.01.001

Nakano N, Kitaura J, 2022. Mucosal mast cells as key effector cells in food allergies. *Cells*, 11(3):329. https://doi.org/10.3390/cells11030329

Niess JH, Adler G, 2010. Enteric flora expands gut lamina propria CX_3CR1^+ dendritic cells supporting inflammatory immune responses under normal and inflammatory conditions. *J Immunol*, 184(4):2026-2037. https://doi.org/10.4049/jimmunol.0901936

Nowak-Węgrzyn A, Albin S, 2015. Oral immunotherapy for food allergy: mechanisms and role in management. *Clin Exp Allergy*, 45(2):368-383. https://doi.org/10.1111/cea.12382

Nowak-Wegrzyn A, Bloom KA, Sicherer SH, et al., 2008. Tolerance to extensively heated milk in children with cow's milk allergy. *J Allergy Clin Immunol*, 122(2):342-347.e2. https://doi.org/10.1016/j.jaci.2008.05.043

Nowak-Węgrzyn A, Wood RA, Nadeau KC, et al., 2019. Multicenter, randomized, double-blind, placebo-controlled clinical trial of vital wheat gluten oral immunotherapy. *J Allergy Clin Immunol*, 143(2):651-661.e9. https://doi.org/10.1016/j.jaci.2018.08.041

Nozawa A, Okamoto Y, Movérare R, et al., 2014. Monitoring Ara h 1, 2 and 3-sIgE and sIgG4 antibodies in peanut allergic children receiving oral rush immunotherapy. *Pediatr Allergy Immunol*, 25(4):323-328. https://doi.org/10.1111/pai.12243

Nucera E, Inchingolo R, Nicotra R, et al., 2021. Influence of antihistamines on basophil activation test in food allergy to milk and egg. *Diagnostics (Basel)*, 11(1):44. https://doi.org/10.3390/diagnostics11010044

Nurmatov U, Dhami S, Arasi S, et al., 2017. Allergen immunotherapy for IgE-mediated food allergy: a systematic review and meta-analysis. *Allergy*, 72(8):1133-1147. https://doi.org/10.1111/all.13124

Ocmant A, Mulier S, Hanssens L, et al., 2009. Basophil activation tests for the diagnosis of food allergy in children. *Clin Exp Allergy*, 39(8):1234-1245. https://doi.org/10.1111/j.1365-2222.2009.03292.x

Orgel K, Burk C, Smeekens J, et al., 2019. Blocking antibodies induced by peanut oral and sublingual immunotherapy suppress basophil activation and are associated with sustained unresponsiveness. *Clin Exp Allergy*, 49(4):461-470. https://doi.org/10.1111/cea.13305

Pajno GB, Bernardini R, Peroni D, et al., 2017. Clinical practice recommendations for allergen-specific immunotherapy in children: the Italian consensus report. *Ital J Pediatr*, 43(1):13. https://doi.org/10.1186/s13052-016-0315-y

Patel G, Saltoun C, 2019. Skin testing in allergy. *Allergy Asthma Proc*, 40(6):366-368. https://doi.org/10.2500/aap.2019.40.4248

Patil SU, Ogunniyi AO, Calatroni A, et al., 2015. Peanut oral immunotherapy transiently expands circulating Ara h 2-specific B cells with a homologous repertoire in unrelated subjects. *J Allergy Clin Immunol*, 136(1):125-134.e12.

https://doi.org/10.1016/j.jaci.2015.03.026

Patil SU, Steinbrecher J, Calatroni A, et al., 2019. Early decrease in basophil sensitivity to Ara h 2 precedes sustained unresponsiveness after peanut oral immunotherapy. *J Allergy Clin Immunol*, 144(5):1310-1319.e4. https://doi.org/10.1016/j.jaci.2019.07.028

Patil SU, Bunyavanich S, Berin MC, 2020. Emerging food allergy biomarkers. *J Allergy Clin Immunol Pract*, 8(8): 2516-2524. https://doi.org/10.1016/j.jaip.2020.04.054

Patriarca G, Schiavino D, Nucera E, et al., 1998. Food allergy in children: results of a standardized protocol for oral desensitization. *Hepatogastroenterology*, 45(19):52-58.

Perezábad L, Reche M, Valbuena T, et al., 2015. Clinical efficacy and immunological changes subjacent to egg oral immunotherapy. *Ann Allergy Asthma Immunol*, 114(6):504-509. https://doi.org/10.1016/j.anai.2015.04.006

Perezábad L, Reche M, Valbuena T, et al., 2017. Oral food desensitization in children with IgE-mediated cow's milk allergy: immunological changes underlying desensitization. *Allergy Asthma Immunol Res*, 9(1):35-42. https://doi.org/10.4168/aair.2017.9.1.35

Perkin MR, Logan K, Tseng A, et al., 2016. Randomized trial of introduction of allergenic foods in breast-fed infants. *N Engl J Med*, 374(18):1733-1743. https://doi.org/10.1056/NEJMoa1514210

Planchais C, Mouquet H, 2020. Easy pan-detection of human IgA immunoglobulins. *J Immunol Methods*, 484-485: 112833. https://doi.org/10.1016/j.jim.2020.112833

Plum T, Wang X, Rettel M, et al., 2020. Human mast cell proteome reveals unique lineage, putative functions, and structural basis for cell ablation. *Immunity*, 52(2):404-416.e5. https://doi.org/10.1016/j.immuni.2020.01.012

Rachid R, Chatila TA, 2016. The role of the gut microbiota in food allergy. *Curr Opin Pediatr*, 28(6):748-753. https://doi.org/10.1097/MOP.0000000000000427

Ramanan D, Sefik E, Galván-Peña S, et al., 2020. An immunologic mode of multigenerational transmission governs a gut Treg setpoint. *Cell*, 181(6):1276-1290.e13. https://doi.org/10.1016/j.cell.2020.04.030

Rivas MN, Burton OT, Wise P, et al., 2015. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity*, 42(3): 512-523. https://doi.org/10.1016/j.immuni.2015.02.004

Rolinck-Werninghaus C, Staden U, Mehl A, et al., 2005. Specific oral tolerance induction with food in children: transient or persistent effect on food allergy? *Allergy*, 60(10): 1320-1322. https://doi.org/10.1111/j.1398-9995.2005.00882.x

Rubio A, Vivinus-Nébot M, Bourrier T, et al., 2011. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children. *Allergy*, 66(1): 92-100. https://doi.org/10.1111/j.1398-9995.2010.02432.x

Ruinemans-Koerts J, Schmidt-Hieltjes Y, Jansen A, et al., 2019. The Basophil Activation Test reduces the need for a food challenge test in children suspected of IgE-mediated cow's milk allergy. *Clin Exp Allergy*, 49(3):350-356. https://doi.org/10.1111/cea.13307

Ruiter B, Shreffler WG, 2012. The role of dendritic cells in food allergy. *J Allergy Clin Immunol*, 129(4):921-928. https://doi.org/10.1016/j.jaci.2012.01.080

Ryan JF, Hovde R, Glanville J, et al., 2016. Successful immunotherapy induces previously unidentified allergen-specific CD4+ T-cell subsets. *Proc Natl Acad Sci USA*, 113(9): E1286-E1295. https://doi.org/10.1073/pnas.1520180113

Sackesen C, Suárez-Fariñas M, Silva R, et al., 2019. A new Luminex-based peptide assay to identify reactivity to baked, fermented, and whole milk. *Allergy*, 74(2):327-336. https://doi.org/10.1111/all.13581

Salamon P, Shefler I, Moshkovits I, et al., 2017. IL-33 and IgE stimulate mast cell production of IL-2 and regulatory T cell expansion in allergic dermatitis. *Clin Exp Allergy*, 47(11):1409-1416. https://doi.org/10.1111/cea.13027

Salmivesi S, Paassilta M, Huhtala H, et al., 2018. Elevated serum adipsin may predict unsuccessful treatment for cows' milk allergy but other biomarkers do not. *Acta Paediatr*, 107(2):328-332. https://doi.org/10.1111/apa.14117

Santos AF, Lack G, 2016. Basophil activation test: food challenge in a test tube or specialist research tool? *Clin Transl Allergy*, 6:10. https://doi.org/10.1186/s13601-016-0098-7

Santos AF, Douiri A, Bécares N, et al., 2014. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J Allergy Clin Immunol*, 134(3): 645-652. https://doi.org/10.1016/j.jaci.2014.04.039

Santos AF, Du Toit G, Douiri A, et al., 2015a. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. *J Allergy Clin Immunol*, 135(1):179-186. https://doi.org/10.1016/j.jaci.2014.09.001

Santos AF, James LK, Bahnson HT, et al., 2015b. IgG_4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol*, 135(5):1249-1256. https://doi.org/10.1016/j.jaci.2015.01.012

Santos AF, Couto-Francisco N, Bécares N, et al., 2018. A novel human mast cell activation test for peanut allergy. *J Allergy Clin Immunol*, 142(2):689-691.e9. https://doi.org/10.1016/j.jaci.2018.03.011

Santos AF, Du Toit G, O'Rourke C, et al., 2020. Biomarkers of severity and threshold of allergic reactions during oral peanut challenges. *J Allergy Clin Immunol*, 146(2):344-355. https://doi.org/10.1016/j.jaci.2020.03.035

Santos AF, Alpan O, Hoffmann HJ, 2021. Basophil activation test: mechanisms and considerations for use in clinical trials and clinical practice. *Allergy*, 76(8):2420-2432.

https://doi.org/10.1111/all.14747

Sanz ML, Maselli JP, Gamboa PM, et al., 2002. Flow cytometric basophil activation test: a review. *J Investig Allergol Clin Immunol*, 12(3):143-154.

Sarfati M, Wakahara K, Chapuy L, et al., 2015. Mutual interaction of basophils and T cells in chronic inflammatory diseases. *Front Immunol*, 6:399. https://doi.org/10.3389/fimmu.2015.00399

Satoguina JS, Weyand E, Larbi J, et al., 2005. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol*, 174(8):4718-4726. https://doi.org/10.4049/jimmunol.174.8.4718

Savilahti EM, Kuitunen M, Savilahti E, et al., 2014a. Specific antibodies in oral immunotherapy for cow's milk allergy: kinetics and prediction of clinical outcome. *Int Arch Allergy Immunol*, 164(1):32-39. https://doi.org/10.1159/000361023

Savilahti EM, Kuitunen M, Valori M, et al., 2014b. Use of IgE and IgG4 epitope binding to predict the outcome of oral immunotherapy in cow's milk allergy. *Pediatr Allergy Immunol*, 25(3):227-235. https://doi.org/10.1111/pai.12186

Schmidt-Weber CB, Blaser K, 2002. T-cell tolerance in allergic response. *Allergy*, 57(9):762-768. https://doi.org/10.1034/j.1398-9995.2002.02158.x

Schwarz A, Panetta V, Cappella A, et al., 2016. IgG and IgG_4 to 91 allergenic molecules in early childhood by route of exposure and current and future IgE sensitization: results from the Multicentre Allergy Study birth cohort. *J Allergy Clin Immunol*, 138(5):1426-1433.e12. https://doi.org/10.1016/j.jaci.2016.01.057

Shakoor Z, al Faifi A, al Amro B, et al., 2016. Prevalence of IgG-mediated food intolerance among patients with allergic symptoms. *Ann Saudi Med*, 36(6):386-390. https://doi.org/10.5144/0256-4947.2016.386

Shamji MH, Kappen J, Abubakar-Waziri H, et al., 2019. Nasal allergen-neutralizing IgG_4 antibodies block IgE-mediated responses: novel biomarker of subcutaneous grass pollen immunotherapy. *J Allergy Clin Immunol*, 143(3):1067-1076. https://doi.org/10.1016/j.jaci.2018.09.039

Shik D, Tomar S, Lee JB, et al., 2017. IL-9-producing cells in the development of IgE-mediated food allergy. *Semin Immunopathol*, 39(1):69-77. https://doi.org/10.1007/s00281-016-0605-x

Shiokawa A, Kotaki R, Takano T, et al., 2017. Mesenteric lymph node $CD11b^-$ $CD103^+$ PD-$L1^{high}$ dendritic cells highly induce regulatory T cells. *Immunology*, 152(1):52-64. https://doi.org/10.1111/imm.12747

Shreffler WG, Lencer DA, Bardina L, et al., 2005. IgE and IgG_4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2. *J Allergy Clin Immunol*, 116(4):893-899. https://doi.org/10.1016/j.jaci.2005.06.033

Shreffler WG, Wanich N, Moloney M, et al., 2009. Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. *J Allergy Clin Immunol*, 123(1):43-52.e7. https://doi.org/10.1016/j.jaci.2008.09.051

Sicherer SH, Sampson HA, 2014. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol*, 133(2):291-307.e5. https://doi.org/10.1016/j.jaci.2013.11.020

Smaldini PL, Delgado MLO, Fossati CA, et al., 2015. Orally-induced intestinal $CD4^+$ $CD25^+$ $FoxP3^+$ Treg controlled undesired responses towards oral antigens and effectively dampened food allergic reactions. *PLoS ONE*, 10(10):e0141116. https://doi.org/10.1371/journal.pone.0141116

Smart JM, Kemp AS, 2002. Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease. *Clin Exp Allergy*, 32(5):796-802. https://doi.org/10.1046/j.1365-2222.2002.01391.x

Smeekens JM, Kulis MD, 2020. Evolution of immune responses in food immunotherapy. *Immunol Allergy Clin North Am*, 40(1):87-95. https://doi.org/10.1016/j.iac.2019.09.006

Song Y, Wang JL, Leung N, et al., 2015. Correlations between basophil activation, allergen-specific IgE with outcome and severity of oral food challenges. *Ann Allergy Asthma Immunol*, 114(4):319-326. https://doi.org/10.1016/j.anai.2015.01.006

Spertini F, 2020. Metabolomics and allergy: opening Pandora's box. *J Allergy Clin Immunol*, 145(3):782-784. https://doi.org/10.1016/j.jaci.2020.01.012

Sporik R, Hill DJ, Hosking CS, 2000. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy*, 30(11):1540-1546. https://doi.org/10.1046/j.1365-2222.2000.00928.x

Stanic B, van de Veen W, Wirz OF, et al., 2015. IL-10-overexpressing B cells regulate innate and adaptive immune responses. *J Allergy Clin Immunol*, 135(3):771-780.e8. https://doi.org/10.1016/j.jaci.2014.07.041

Suárez-Fariñas M, Suprun M, Chang HL, et al., 2019. Predicting development of sustained unresponsiveness to milk oral immunotherapy using epitope-specific antibody binding profiles. *J Allergy Clin Immunol*, 143(3):1038-1046. https://doi.org/10.1016/j.jaci.2018.10.028

Sugimoto M, Kamemura N, Nagao M, et al., 2016. Differential response in allergen-specific IgE, IgGs, and IgA levels for predicting outcome of oral immunotherapy. *Pediatr Allergy Immunol*, 27(3):276-282. https://doi.org/10.1111/pai.12535

Suprun M, Sicherer SH, Wood RA, et al., 2020. Early epitope-specific IgE antibodies are predictive of childhood peanut allergy. *J Allergy Clin Immunol*, 146(5):1080-1088. https://doi.org/10.1016/j.jaci.2020.08.005

Syed A, Garcia MA, Lyu SC, et al., 2014. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (*FOXP3*). *J Allergy Clin Immunol*, 133(2):500-510.e11. https://doi.org/10.1016/j.jaci.2013.12.1037

Takasato Y, Kurashima Y, Kiuchi M, et al., 2021. Orally desensitized mast cells form a regulatory network with Treg cells for the control of food allergy. *Mucosal Immunol*, 14(3):640-651. https://doi.org/10.1038/s41385-020-00358-3

Tang MLK, Ponsonby AL, Orsini F, et al., 2015. Administration of a probiotic with peanut oral immunotherapy: a randomized trial. *J Allergy Clin Immunol*, 135(3):737-744.e8. https://doi.org/10.1016/j.jaci.2014.11.034

The Medical Letter, 2020. Peanut allergen powder (PalfoPrzia). *JAMA*, 324(2):192-193. https://doi.org/10.1001/jama.2020.3599

The PALISADE Group of Clinical Investigators, 2018. AR101 oral immunotherapy for peanut allergy. *N Engl J Med*, 379(21):1991-2001. https://doi.org/10.1056/NEJMoa1812856

Thota LN, Ponnusamy T, Philip S, et al., 2017. Immune regulation by oral tolerance induces alternate activation of macrophages and reduces markers of plaque destabilization in Apob^{tm2Sgy}/Ldlr$^{tm1Her/J}$ mice. *Sci Rep*, 7:3997. https://doi.org/10.1038/s41598-017-04183-w

Tordesillas L, Berin MC, 2018. Mechanisms of oral tolerance. *Clin Rev Allergy Immunol*, 55(2):107-117. https://doi.org/10.1007/s12016-018-8680-5

Tordesillas L, Mondoulet L, Blazquez AB, et al., 2017. Epicutaneous immunotherapy induces gastrointestinal LAP$^+$ regulatory T cells and prevents food-induced anaphylaxis. *J Allergy Clin Immunol*, 139(1):189-201.e4. https://doi.org/10.1016/j.jaci.2016.03.057

Torgerson TR, Linane A, Moes N, et al., 2007. Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the *FOXP3* gene. *Gastroenterology*, 132(5):1705-1717. https://doi.org/10.1053/j.gastro.2007.02.044

Tsai M, Mukai K, Chinthrajah RS, et al., 2020. Sustained successful peanut oral immunotherapy associated with low basophil activation and peanut-specific IgE. *J Allergy Clin Immunol*, 145(3):885-896.e6. https://doi.org/10.1016/j.jaci.2019.10.038

Tsuji M, Komatsu N, Kawamoto S, et al., 2009. Preferential generation of follicular B helper T cells from Foxp3$^+$ T cells in gut peyer's patches. *Science*, 323(5920):1488-1492. https://doi.org/10.1126/science.1169152

Turcanu V, Brough HA, Du Toit G, et al., 2017. Immune mechanisms of food allergy and its prevention by early intervention. *Curr Opin Immunol*, 48:92-98. https://doi.org/10.1016/j.coi.2017.08.009

Uranüs S, Kronberger L, Neumayer K, et al., 1990. TA-stapler resection of congenital splenic cyst. Case report. *Acta Chir Scand*, 156(3):247-249.

van de Veen W, Stanic B, Yaman G, et al., 2013. IgG_4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *J Allergy Clin Immunol*, 131(4):1204-1212. https://doi.org/10.1016/j.jaci.2013.01.014

van de Veen W, Stanic B, Wirz OF, et al., 2016. Role of regulatory B cells in immune tolerance to allergens and beyond. *J Allergy Clin Immunol*, 138(3):654-665. https://doi.org/10.1016/j.jaci.2016.07.006

Varol C, Vallon-Eberhard A, Elinav E, et al., 2009. Intestinal lamina propria dendritic cell subsets have different origin and functions. *Immunity*, 31(3):502-512. https://doi.org/10.1016/j.immuni.2009.06.025

Varshney P, Jones SM, Scurlock AM, et al., 2011. A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. *J Allergy Clin Immunol*, 127(3):654-660. https://doi.org/10.1016/j.jaci.2010.12.1111

Vazquez-Ortiz M, Pascal M, Alsina L, et al., 2013. Serum sIgG4, but not sIgA, is involved in induced and natural tolerance to egg allergens. *Clin Transl Allergy*, 3(3):O6. https://doi.org/10.1186/2045-7022-3-S3-O6

Vickery BP, Pons L, Kulis M, et al., 2010. Individualized IgE-based dosing of egg oral immunotherapy and the development of tolerance. *Ann Allergy Asthma Immunol*, 105(6):444-450. https://doi.org/10.1016/j.anai.2010.09.030

Vickery BP, Lin J, Kulis M, et al., 2013. Peanut oral immunotherapy modifies IgE and IgG_4 responses to major peanut allergens. *J Allergy Clin Immunol*, 131(1):128-134.e3. https://doi.org/10.1016/j.jaci.2012.10.048

Vickery BP, Scurlock AM, Kulis M, et al., 2014. Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy. *J Allergy Clin Immunol*, 133(2):468-475.e6. https://doi.org/10.1016/j.jaci.2013.11.007

Vickery BP, Berglund JP, Burk CM, et al., 2017. Early oral immunotherapy in peanut-allergic preschool children is safe and highly effective. *J Allergy Clin Immunol*, 139(1):173-181.e8. https://doi.org/10.1016/j.jaci.2016.05.027

Vivier E, Artis D, Colonna M, et al., 2018. Innate lymphoid cells: 10 years on. *Cell*, 174(5):1054-1066. https://doi.org/10.1016/j.cell.2018.07.017

Vonk MM, Diks MAP, Wagenaar L, et al., 2017. Improved efficacy of oral immunotherapy using non-digestible oligosaccharides in a murine cow's milk allergy model: a potential role for Foxp3+ regulatory T cells. *Front Immunol*, 8:1230. https://doi.org/10.3389/fimmu.2017.01230

Vonk MM, Blokhuis BRJ, Diks MAP, et al., 2019. Butyrate enhances desensitization induced by oral immunotherapy in cow's milk allergic mice. *Mediators Inflamm*, 2019:9062537. https://doi.org/10.1155/2019/9062537

Wai CYY, Leung NYH, Leung ASY, et al., 2021. Cell-based functional IgE assays are superior to conventional allergy tests for shrimp allergy diagnosis. *J Allergy Clin Immunol Pract*, 9(1):236-244.e9. https://doi.org/10.1016/j.jaip.2020.08.057

Wambre E, Delong JH, James EA, et al., 2012. Differentiation stage determines pathologic and protective allergen-specific CD4$^+$ T-cell outcomes during specific immunotherapy. *J Allergy Clin Immunol*, 129(2):544-551.e7. https://doi.org/10.1016/j.jaci.2011.08.034

Wambre E, Bajzik V, Delong JH, et al., 2017. A phenotypically

and functionally distinct human T_H2 cell subpopulation is associated with allergic disorders. *Sci Transl Med*, 9(401): eaam9171. https://doi.org/10.1126/scitranslmed.aam9171

Wang S, Xia PY, Chen Y, et al., 2017. Regulatory innate lymphoid cells control innate intestinal inflammation. *Cell*, 171(1):201-216.e18. https://doi.org/10.1016/j.cell.2017.07.027

Wang YG, Ding YX, Guo NZ, et al., 2019. MDSCs: key criminals of tumor pre-metastatic niche formation. *Front Immunol*, 10:172. https://doi.org/10.3389/fimmu.2019.00172

Welty NE, Staley C, Ghilardi N, et al., 2013. Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. *J Exp Med*, 210(10):2011-2024. https://doi.org/10.1084/jem.20130728

Wright BL, Kulis M, Orgel KA, et al., 2016. Component-resolved analysis of IgA, IgE, and IgG4 during egg OIT identifies markers associated with sustained unresponsiveness. *Allergy*, 71(11):1552-1560. https://doi.org/10.1111/all.12895

Yamashita H, Matsuhara H, Miotani S, et al., 2017. Artificial sweeteners and mixture of food additives cause to break oral tolerance and induce food allergy in murine oral tolerance model for food allergy. *Clin Exp Allergy*, 47(9): 1204-1213. https://doi.org/10.1111/cea.12928

Yee LM, Lively TG, McShane LM, 2018. Biomarkers in early-phase trials: fundamental issues. *Bioanalysis*, 10(12):933-944. https://doi.org/10.4155/bio-2018-0006

Yoneyama T, Nakano N, Hara M, et al., 2021. Notch signaling contributes to the establishment of sustained unresponsiveness to food allergens by oral immunotherapy. *J Allergy Clin Immunol*, 147(3):1063-1076.e9. https://doi.org/10.1016/j.jaci.2020.07.011

Zhou L, Chu C, Teng F, et al., 2019. Innate lymphoid cells support regulatory T cells in the intestine through interleukin-2. *Nature*, 568(7752):405-409. https://doi.org/10.1038/s41586-019-1082-x