An update on recent developments and highlights in food allergy

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Abstract

While both the incidence and general awareness of food allergies is increasing, the variety and clinical availability of therapeutics remain limited. Therefore, investigations into the potential factors contributing to the development of food allergy and the mechanisms of natural tolerance or induced desensitization are required. In addition, a detailed understanding of the pathophysiology of food allergies is needed to generate compelling, enduring, and safe treatment options. New findings regarding the contribution of barrier function, the effect of emollient interventions, mechanisms of allergen recognition, and the contributions of specific immune cell subsets through rodent models and human clinical studies provide novel insights. With the first approved treatment for peanut allergy, the clinical management of food allergy is evolving towards less intensive, alternative approaches involving fixed doses, lower maintenance dose targets, co-administration of biologicals, adjuvants, and tolerance-inducing formulations. The ultimate goal is to improve immunotherapy and develop precision-based medicine via risk phenotyping allowing optimal treatment for each food-allergic patient.

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List of Abbreviations:

Allergen immunotherapy (AIT)

Antigen presenting cells (APCs)

Atopic dermatitis (AD)

Epicutaneous immunotherapy (EPIT)

Fecal microbiota transplant (FMT)

Food allergy (FA)

Gastrointestinal tract (GIT)

G-protein-coupled receptor (GPCR)

Innate lymphoid cells (ILCs)

Oral immunotherapy (OIT)

Peanut allergic (PA)

Regulatory ILCs (ILCregs)

Regulatory T cells (Treg)

Short-chain fatty acids (SCFAs)

Subcutaneous immunotherapy (SCIT)

Sublingual immunotherapy (SLIT)

T-follicular helper cell (Tfh)

Type 1 regulatory T cells (Tr1)

Abstract

While both the incidence and general awareness of food allergies is increasing, the variety and clinical availability of therapeutics remain limited. Therefore, investigations into the potential factors contributing to the development of food allergy and the mechanisms of natural tolerance or induced desensitization are required. In addition, a detailed understanding of the pathophysiology of food allergies is needed to generate compelling, enduring, and safe treatment options. New findings regarding the contribution of barrier function, the effect of emollient interventions, mechanisms of allergen recognition, and the contributions of specific immune cell subsets through rodent models and human clinical studies provide novel insights. With the first approved treatment for peanut allergy, the clinical management of food allergy is evolving towards less intensive, alternative approaches involving fixed doses, lower maintenance dose targets, co-administration of biologicals, adjuvants, and tolerance-inducing formulations. The ultimate goal is to improve immunotherapy and develop precision-based medicine via risk phenotyping allowing optimal treatment for each food-allergic patient.

Introduction

We provide an update from our previous manuscript[1]covering recent advances in the field with specific focus on biomarkers of desensitization and tolerance development.

1.1 Food allergy and barrier function

At the interface between the "exposome" and the human body, epithelial cells act as the first physical barrier of protection and play an integral part in maintaining tissue homeostasis. The exposome comprises external factors including allergens, pollutants, detergents and microbes, and internal factors namely the microbiota and metabolic products.[2] Maintaining proper barrier function is crucial for facilitating appropriate immune responses to allergens (Figure 1). Consequently, epithelial barrier dysfunction and altered permeability caused by gene mutations or single nucleotide polymorphisms on key genes including filaggrin, SPINK5[3], SERPINB7, KLK7 and Claudin-1[4,5] are associated with atopic dermatitis (AD) and food allergy (FA) development.[6–10] Barrier abnormalities arise from decreases in ceramides, antimicrobial peptides, serine protease, and skin/gut microbiome dysbiosis through exogenic factors, namely detergents.[4,11,12] A recent review in*Allergy* explores these concepts further.[13]

AD is associated with FA development through the dual-allergen exposure hypothesis, which suggests primary allergen exposure through damaged skin without prior gastrointestinal tract (GIT) exposure favours an inflammatory Th2-type immune response. In contrast, initial allergen exposure through the GIT promotes regulatory immune responses and tolerance induction.[14,15] Consequently, food allergen sensitization and FA development is likely linked to skin barrier dysfunction and potentially microbial colonization.[4,15–17] Commensal bacteria are needed for skin microbiota protection, maturation of T cells and activation of antimicrobial peptide production by keratinocytes. Dysbiosis in the skin microbiome, commonly measured by *Staphylococcus aureus* abundance, is positively correlated with AD severity, serving as a promising AD biomarker.[18,19]

The use of emollients is the main AD management strategy with the intent to support barrier function. This concept of preventing barrier dysfunction *in vivo* via the provision of moisturizers is a topic of ongoing investigation. Various formulations of emollients, including the most common paraffin/petroleum base, and a trilipid base (3:1:1- ceramides, cholesterol and free fatty acids) are used.[20,21] Emollients may reduce severity and prolong the time between AD flares[22] by reducing skin water loss[21] and improving overall hydration.[23] This is crucial for neonatal skin which is characterized by a thin stratum corneum layer with reduced lipid content and moisturizing factors. Emollients, specifically the trilipid formulation, may promote tolerance with an increased IgG4/IgE ratio and IL-10, LAP^+ T cells and decreased IL-4 producing $CD4^+$ T cells.[20] Additionally, free fatty acids present in the trilipid formulation may activate peroxisome proliferator-activated receptors (PPARs), which are generally reduced in AD, and thereby reduce inflammation.[24]

Initial pilot studies demonstrated AD could be prevented by regular emollient use.[20,25] However, larger randomized controlled trials like BEEP[26] a multicentre trial with high-risk-of-allergy newborns concluded daily application of paraffin/petroleum-based emollient did not prevent eczema at 2 years of age or reduce incidence of FA despite good treatment adherence.[26] The PreventADALL[27]trial conducted on newborns, reaffirmed neither intensive emollient use (paraffin/petroleum-based and emollient bath oil) nor early complementary feeding reduced AD development.[27]Allergic sensitization at 6 months was predicted by eczema, dry skin and impaired skin barrier function at 3 months of age.[28]However, the emollient formulations may be relevant and additional large population studies investigating the efficacy of different emollients in AD treatment are ongoing.[25,29–31]

1.2 Updates on the humoral mechanisms of allergen recognition

Allergen recognition by IgE and receptor crosslinking are central to the initiation of the acute allergic response. For IgE to elicit its effector functions, glycosylation in the constant region of the antibody plays

a pivotal role. The presence of an oligomannose glycan at the N394 residue in human IgE is necessary for correct structure and FceRI receptor binding in order to trigger anaphylaxis.[32]Peanut allergic (PA) individuals produced IgE antibodies with greater sialylation content compared to non-allergic individuals, and sialylation enhanced the potency of IgE crosslinking-induced degranulation *in vivo* .[33,34] Therefore, sialylation of IgE may provide an additional diagnostic marker for allergy.

Glycosylation on other isotypes including IgG may contribute to the conferral of early-life atopic predispositions. During healthy pregnancy, antibodies with di-galactosylated glycans in the Fc domain are selectively transferred across the placenta and contribute to early innate immune responses through the induction of natural killer cell activity.[35] In the context of asthma, mice experiencing exacerbations during pregnancy had higher levels of a pro-inflammatory glycosylation pattern, as reflected by the absence of galactose and sialic acid end branches on the Fc part of IgG1. Maternal glycosylation patterns were correlated with patterns observed in offspring later developing allergic asthma.[36] The importance of glycosylation for IgG functionality is supported by discordant IgG Fc glycosylation patterns identified between healthy children and children with recurrent respiratory infections.[37]

An important factor determining IgE's potency to elicit clinical reactions is affinity. Affinity maturation in germinal centers is guided by T-follicular helper (Tfh) cells, which promote the selection and expansion of B cells.[38] The affinity of IgE may be specifically affected by Tfh cells. A newly identified IL-13 producing Tfh13 cell was required to produce high but not low-affinity IgE and subsequent allergen-induced anaphylaxis in a murine model. Tfh13 cells display a characteristic cytokine profile (IL-13hiIL4hiIL-5hiIL-21lo), co-express transcription factors BCL6 and GATA3 and are more abundant in PA individuals.[39]

Characteristics of epitope recognition influence the strength of an allergic reaction via the diversity of epitopes, their abundance[40], proximity, and overlap with other isotypes (e.g., IgG4).[41,42] Linear epitopes to peanut allergens Ara h 1-11 were recently compared between PA and sensitized, non-allergic individuals.[43] Seven peptides from the seed storage proteins Ara h 1, 2 and 3 were preferentially recognized by IgE from PA individuals, while the IgG4:IgE ratio was higher in peanut sensitized non-allergics compared to PA individuals, indirectly suggesting a functional role of IgG4 in tolerance development.[43](Figure 2) Ara h 2 sIgE is known to enhance diagnostic accuracy and is superior to extract-based methods.[44–47] Machine-learning approaches in early life (3-15 months) suggested the IgE peanut epitope repertoire was predictive of the development of PA at 4 years.[48]Bead-based assays used in the context of egg allergy showed egg allergic children had higher levels of epitope-specific IgE and IgD and lower IgA and IgG to Gal d 1 than atopic controls.[49,50] Thus, continued research on linear and conformational IgE epitopes which includes various isotypes could expand the toolkit to predict the progression of atopic disease and responses to therapeutic interventions.

Local antibody production via reservoirs of IgE+ B cell lineages in the GIT undergoing local class switching may be crucial. Sequential biopsies in the upper GIT revealed increased numbers for IgE+ B lineage cells in the mucosa of PA patients, which correlated with systemic peanut sIgE levels. B cell clonal lineages within biopsies were comprised of both IgE+ and non-IgE+ isotypes, suggesting class switch recombination can occur locally.[51] Thus, the GIT could serve as an important reservoir for allergen-specific plasma cells later found in other tissues.[51]

1.3 Updates on the cellular network in food allergy

The innate immune system is a rapid-onset, non-specific defense mechanism upstream of adaptive inflammatory allergic responses. The major effector cells include basophils and mast cells, which release histamine and other pre-formed inflammatory mediators following allergen exposure. Basophils are key players in the pathophysiology of FA.[52] They produce large quantities of IL-4, facilitating mast cell recruitment, activation, proliferation and isotype switching of B cells to IgE antibody production.[53] Basophils derived from allergic individuals showed enhanced sensitivity to IL-1β and IL-33 compared to healthy controls.[54] The alarmin IL-33, which mediates inflammation of mucosal and epithelial surfaces upon exposome interactions, is a key

upstream component of type 2 responses and a potent activator of mast cells. Recently, the first human mutation in the IL-33 gene was described, showing a complex phenotype of type 2 inflammation-dominated immune dysregulation.[55] IL-33 and IgE-mediated activation of mast cells suppressed regulatory T cell (Treg) conversion from naïve T cells in a murine co-culture model.[56] Similarly, a murine knockout of CD300f, an inhibitory receptor on mast cells, exhibited reduced Treg numbers and exacerbated allergic responses.[57] These studies suggest blocking IL-33 and IgE during OIT may be beneficial in reducing allergic responses by downregulating mast cell activation and promoting the generation of Tregs.[56] Correspondingly, a small clinical study reported a single dose of the anti-IL-33 biologic Etokimab was shown to elevate the threshold to peanuts in allergic patients, reduce allergen specific type 2 cell frequencies or cytokine production (IL-4, 5, 9 and 13) and peanut-specific IgE.[58]

Mast cell contribution to the development of allergic diseases is traced back to early life. In rodents, fetal mast cells can be sensitized with maternal allergen-specific IgE and showed allergen sensitivity during postnatal exposure.[59] A human *ex vivo* placental perfusion model demonstrated functional peanut allergen could also be transferred across the placenta. However, evidence for the transfer of IgE even with omalizumab treatment could not be provided, despite the potential for IgG-mediated transport.[60] The relevance of these findings for the human system remains to be established since they challenge the concept IgE is not transferred to the fetus.[59,60]

Rare subsets such as innate lymphoid cells (ILCs) are enriched at mucosal sites and are crucial in initiating and regulating allergic responses. ILC2s are capable of rapidly secreting pro-allergenic cytokines such as IL-5, -4, -13 and -9 upon activation by alarmins (IL-33, IL-25 and TSLP). ILC2-derived IL-13 contributes to Tfh cell development and allergen-specific IgE generation.[61,62] Recently, the regulatory capacity of ILC2 cells in controlling inflammation was recognized. It is not clear whether there is a distinct group of regulatory ILCs (ILCregs) or a subset of ILC2s producing tolerogenic IL-10. These ILCreg cells were identified in human intestines and kidneys suppressing inflammation and activation of ILC1/3 cells via IL-10 and TGF-β1 production.[63,64] Following allergen immunotherapy (AIT), IL-10$^+$ ILC2 cells were upregulated in the allergic group, suggesting these cells may be involved in tolerance development.[65]

T and B cells are drivers of the adaptive immune system responsible for the generation of allergen-specific memory. The Generation R cohort reported children with atopic diseases had a higher proportion of Th2, Th17, Treg, memory Treg, and CD27$^+$IgA$^+$ memory B cells compared to non-atopic children, which may point toward important regulatory processes initiated during allergic disease.[66] PA patients with a lower tolerance and high clinical sensitivity have a larger, more diversified allergen-specific CD4+ T cell repertoire compared to hyporeactive patients. This repertoire is enriched with Th2-skewed effector T cells and more responsive to allergen-stimulation.[67] Highly reactive patients also have a higher frequency of peanut-specific Th2a cells.[67] This pro-inflammatory subset is characterized by CRTH2, CD161 and CD49d expression and co-secretion of type-2 cytokines.[68] Th2a cells are linked to the pathogenesis of atopic disease, and decreased significantly in allergic patients treated with AIT.[68] Suppression of Th2a-like cells was associated with better treatment outcomes post-OIT.[69]In a Phase 2 clinical trial (NCT02626611) of multi-food-OIT under omalizumab protection, there was a significant decrease in Th2a and Th17 cells in participants >10-years-old. DCreg markers STAB1 and FcγRIIIa were also significantly altered. Therefore, Th2a and Th17 cell frequencies and DCreg markers could expand the toolkit of biomarkers to monitor successful OIT.[70]

Tregs are proposed to be key cellular mediators of tolerance induction.[71,72] Lower proportions of naïve Tregs at birth and in cord blood are predictors of FA development in infancy.[73,74] There are several Treg subtypes involved in tolerance: FoxP3$^+$ Tregs, TGF-β secreting Th3 cells and IL-10 secreting type 1 regulatory T (Tr1) cells. Tr1 cell populations were found to be significantly higher in younger (<6-years-old), non-allergic children compared to food allergic children. These Tr1 cells expressed higher levels of CCR6, a gut-homing marker, indicating a role in promoting local tolerance.[75] Adaptive immune cells play a fundamental role in the development of FA, but also in the induction of desensitization and tolerance.

1.4 Lessons from Rodent Data on Food Allergy

Recent murine studies revealed novel mechanisms employed by the microbiome to promote immune tolerance to food antigens or drive allergic responses resulting in anaphylaxis. Fecal microbiota transplant (FMT) from healthy or food allergic children into germ-free mice was performed by several investigators. Germ-free mice colonized with bacteria from healthy, but not cow's milk allergic infants were protected against anaphylactic responses to a milk allergen, which correlated with distinct transcriptome signatures in the ileal epithelium. One clostridial species, *Anaerostipes caccae* , was associated with protection from an allergic response to food.[76] An independent study replicated these FMT findings and further demonstrated transfer of an infant microbiota with a low bifidobacteria/lachnospiraceae ratio orients the murine immune system toward a Th2 atopic profile with enhanced symptoms of allergy in the murine recipient.[77]

Secretion of metabolites by the microbiome mediate many of the observed functional effects on immune cells within the intestine. Several recent studies have focused on short-chain fatty acids (SCFAs), including butyrate, propionate and acetate. SCFAs were shown to support Treg polarization and expansion.[78] Recently, SCFAs triggering G-protein-coupled receptors (GPCR) were shown to synergize with cytokine receptor signaling to provide key signals to expand tissue populations of ILC1, ILC2 and ILC3.[79] Propionate and butyrate, but not acetate, inhibited IgE- and non–IgE-mediated mast cell degranulation. These effects were independent of the stimulation of SCFA receptors GPR41, GPR43, or PPAR, but instead were associated with inhibition of histone deacetylases.[80,81] Additionally, pre-treatment of mice with butyrate significantly reduced allergic response in three different animal models of FA. This was associated with induction of tolerogenic cytokines, inhibition of Th2 cytokine production and modulation of oxidative stress.[82] GPR109A, which is a receptor for butyrate and niacin, was also shown to be important for maintenance of epithelial function and as a negative regulator of type 2 immune responses.[83] Lastly, a wide range of immunomodulatory bacterial-derived metabolites that activate GPCRs, aryl hydrocarbon receptor and nuclear receptors have recently been described and are currently being examined in murine models of FA.[84]

Murine studies are helping us to better understand the contribution of novel immune cell subsets to FA. In the small intestine, ILC3s, the dominant cellular source of IL-2, are essential for maintaining Tregs, immunological homeostasis, and oral tolerance to dietary antigens. IL-2 is induced selectively by IL-1β, which is produced by macrophages in an MYD88- and NOD2-dependent mechanism.[85] The role of Tfh cells in the induction of peanut-specific IgA was clarified. While IgG1 and IgE responses to peanut require Tfh cells, IgA responses were Tfh-independent suggesting the cellular mechanisms for induction of IgA to food antigens is different to those culminating in IgE or IgG1.[86] Interestingly, mice sensitized to peanut in the absence of an adjuvant were characterized by the presence of increased numbers of Tfh cells in the mesenteric lymph nodes, reduced fecal IgA levels, altered gut permeability and a distinct microbiome composition.[87] Further studies in genetically susceptible mouse strains indicated genetic loci outside of Tlr4 and Dock8 are responsible for the oral anaphylactic susceptibility of C3H/HeJ mice to peanut.[88] The mechanisms via which SCFAs support connective tissue mast cell maturation from immature mouse bone marrow-derived mast cells was demonstrated to involve the transcriptional upregulation of heparin sulfate biosynthesis enzymes, certain mast cell-specific proteases, MAS-related GPCR family members, and transcription factors required for mast cell lineage determination.[89]

2 Mechanisms and potential biomarkers of food allergy immunotherapy

AIT is currently the most promising and researched FA treatment. However, depending on the approach, limitations regarding safety, efficacy, time of up-dosing, costs and the extent of protection arise. Data on sustained unresponsiveness, considered a surrogate of tolerance development, is challenging to obtain. Despite significant improvements in the therapeutic concepts, alternative strategies with better safety profiles capable of inducing tolerance are needed. A variety of different administration routes and doses are being explored: oral (OIT), epicutaneous (EPIT), sublingual (SLIT) and subcutaneous (SCIT). AIT is mechanistically proposed to have its effects when a relevant allergen either in its native or modified form is introduced

starting at a subthreshold level then at incremental doses to induce desensitization and increase the threshold of tolerance. (Figure 3)

OIT is the most common form of AIT (typical maintenance dose 125mg–4g).[90] Via the ingestion of subthreshold amounts of allergen, reduction in allergen-induced basophil and mast cell activation are among the first immunological changes observed. During the initial up-dosing, an upregulation of sIgE production and a transient increase in allergen-specific type 2 effector cells are observed in concurrence with desensitization.[90–93] A transient increase in TGF-β producing CD4+ T cells is also associated with successful desensitization. In adult patients undergoing peanut OIT, transient esophageal eosinophilia and gastrointestinal eosinophilia may occur.[94] As immunotherapy continues, Th2 cell activity and frequency decreases while IL-10 producing regulatory cells (Tregs and Bregs) become more prominent.[70,95] This later phase is associated with an increase in sIgG, sIgG4 and sIgA, and lower sIgE/total IgE ratios.[90,93,96] However, measurement of sIgA as a surrogate for mucosal response does not seem to predict sustained tolerance or successful desensitization.[97] Initiation of OIT before 4 years of age and lower sIgE at baseline are correlated with increased chances of desensitization and remission following OIT.[98] Tissue-specific effects of OIT in humans are under-researched and may play an important role.[99,100]

SLIT utilizes a hundred-fold lower dose of allergen (maintenance dose 2-5mg/day). It takes advantage of local oral tolerance mechanisms as reported for inhalant allergen SLIT.[101,102] Such mechanisms include the uptake of allergens by oral antigen presenting cells (APCs) from the sublingual mucosa. In murine models, $CD103^{-}CD11b^{+}$ DC were implicated in the transfer of antigens to submandibular lymph nodes, which supported Treg differentiation through the production of retinoic acid.[103,104] During the early stages of SLIT treatment, the transient increase in sIgE was attributed to the induction of class switching of IgG+ memory B cells into short-lived IgE+ plasmablasts.[105] Importantly, increases in sIgE from allergen exposure due to SLIT were not associated with a diversification of the IgE repertoire.[105] SLIT was recently investigated for the treatment of birch pollen-related FA using recombinant Mal d 1, which reduced Th2 cell frequency and IL-4 production[106] and increased IgG with functional blocking activity.[107] Additional biomarkers for SLIT include salivary sIgA, which is thought to inhibit allergen uptake at the mucosal surface and demonstrated alignment with treatment outcomes following peanut SLIT.[108] A long-term, five-year peanut SLIT trial reported treatment was associated with decreased sIgE and SPT wheal size, increased IgG4/IgE ratio, and reduced basophil activation.[109]

EPIT directly targets immune cells in the skin. Desensitization is induced through the application of a skin patch with very low doses of allergen in the microgram range (50-250μg).[102,110] The allergen is taken up through the stratum corneum by Langerhans cells, which migrate to lymph nodes and induce Foxp3+ Treg differentiation.[111] Additionally, gut-homing $LAP^{+}FoxP3^{-}$ Tregs are induced and decrease the risk of anaphylaxis via TGF-β-dependent inhibition of mast cell activation.[112] Even though EPIT is shown to be tolerable and safe, its success in provoking desensitisation remains unclear. The phase 3 PEPITES trial for PA successfully demonstrated safety, but did not meet its pre-specified efficacy outcome.[113] The follow-up PEOPLE trial, a 2-year open label extension of the PEPITES study, reported continued EPIT therapy remained clinically beneficial and tolerable, with an increase in eliciting dose from baseline.[114] EPIT continues to be investigated for various allergens including cashew, where EPIT recently demonstrated efficacy in reducing mast cell reactivity and anaphylactic symptoms in mouse models.[115] A range of different types of biomarkers are being utilized to mark the success of the various AIT administration routes. (Table 1)

3 Updates on clinical treatment approaches

The management of FA during the last three years was influenced by the COVID-19 pandemic[116–119] with a shift to telemedicine[120] which will continue to influence practice. OIT has continued to gain momentum as an alternative to food avoidance in the management of FA. The USA[121] and Europe recently approved the first pharmaceutical product for the treatment of PA in children aged 4-17 years. Approaches in other countries[122,123] support the use of foods for OIT. Debate about the best application of OIT continues, with concerns about the risk of severe allergic reactions due to OIT itself needing to be balanced against

possible reduction in reactions from accidental exposure (Figure 4). Mathematical modeling suggests food desensitization is expected to have some benefit in reducing accidental exposure,[124,125] which will vary depending on the increase in threshold achieved. A recent meta-analysis concluded there were less severe allergic reactions in OIT as compared to placebo.[126]

It is increasingly clear OIT must be continued in most patients to maintain its effect, as illustrated in the POISED[127], POIMD[128] and IMPACT[98] studies. After achieving a maintenance dose of 4g/day of peanut protein, 120 children and adults in the POISED study were randomized to no peanut or 300mg peanut and followed over time.[127] Most lost their desensitization and even the ongoing ingestion of 300mg resulted in a lower threshold than when ingesting 4g a day.[127] In the POIMD study, one month of peanut avoidance after peanut OIT lowered the maximum tolerated amount of peanut by an average of more than 7g.[128] Similarly, in the IMPACT study, 26 weeks of peanut avoidance after peanut OIT resulted in only 21% of the treatment group maintaining remission to peanut.[98]

Since OIT is needed long-term for most food allergic individuals, there is significant interest in the dose and the dosing schedule which balances risks and benefits. Slow up-dosing regimens, meaning less frequently than every 2 weeks, appear to reduce adverse events versus quicker regimens.[129] There are now multiple clinical studies aiming to directly assess safety and efficacy of lower doses. In nut allergy for example, NCT04415593, NCT03799328, NCT03532360, and NCT03907397 will focus on dose. Another important aspect is cross protection in tree nut OIT. In a recent study, desensitization to cashew resulted in cross-desensitization to pistachio in patients with a co-allergy, indicating co-treatment of multiple nut allergies with one nut may also be possible.[130]

An even simpler implementation of OIT may be with no up-dosing. Miura et al. reported the outcome of fixed dosing for milk OIT at 1,2,3 years.[131] Children with severe milk allergy received a fixed dose of only 3ml daily with OFC to 25ml each year with 27%, 52%, and 61% achieving this goal. Baseline sIgE levels predicted this success and participants showed significant reduction in sIgE to casein with increased milk and casein specific IgG4. There was no placebo group, but historical controls showed no significant laboratory changes over the same period. Less intensive regimens have the potential benefit of making FA treatment far more accessible and equitable.[132]

For some patients, food desensitization may benefit from the addition of biologicals as adjunctive therapy.[133–135] Currently, multiple studies are using omalizumab or dupilumab either alone or in combination with OIT (NCT04045301, NCT03679676, NCT03881696, and NCT04037176). Although there was interest in the effect of probiotic adjuvants on OIT, the effectiveness of these additions remains to be seen.[136]

Alternative routes to oral food desensitization continue to be studied. SLIT is showing significant efficacy with minimal serious side effects. However, this approach resulted in a high dropout rate.[137,138] The significant reduction in risk and potential to be implemented on a larger scale requires further research.

While EPIT has shown favorable safety and tolerability, its desensitization effect is uncertain.[110] EPIT in 4–12-year-old children found no episodes of severe anaphylaxis and only 4/294 drop-outs.[139] Long-term follow-up showed a stable desensitization effect from 52 weeks to 130 weeks but with no additional desensitization after the one year of treatment.[140] Sub-analysis suggesting a better effect in younger children (1-3 years-old) may provide more insight (NCT03211247, NCT03859700).

There is an emerging change in the clinical management of FA beyond immunotherapy to include non-complete avoidance of the food. For example, the 2020 Japanese Food Allergy Guidelines[141]state the purpose of the OFCs is not only diagnosis of FA, but for determination of the safe quantity for ongoing ingestion. When patients are not highly allergic (e.g., food pollen syndrome, exercise-induced anaphylaxis, baked milk/egg diets) it is widely accepted clinical practice to allow low amounts of the allergenic food in the diet.[142] Overall, these approaches are based in the appreciation that most food allergic individuals do not have an exquisitely severe allergy and can tolerate small amounts of allergen. However, further understanding of dose thresholds[143–146] along with product labeling is needed. Sub-threshold amounts of the allergic food in the diet is encountered in the use of baked milk/egg ladders[147], although it is not yet clear if incorporating

baked milk/egg into the diet of milk and egg allergic individuals is preferred to OIT. Children allergic to unbaked egg but tolerant to baked egg treated with egg OIT were significantly more likely to achieve sustained unresponsiveness in a two-year time frame than children ingesting baked egg.[148] Children allergic to the baked forms can have severe allergic reactions and patients must be chosen carefully for the introduction of baked foods based on negative OFCs.

Matching the patient to the right treatment requires consideration of their risks and benefits in a patient-centered manner. The assessment of risk is hindered by many factors, including inconsistencies in the definition of FA severity (Figure 4). The Consensus on DEfinition of Food Allergy SEverity (DEFACE) initiative[149] aims to standardize severity. The recognition of knowledge gaps in FA management increased focus on shared decision-making to have bi-directional discussions on patients' values, goals, risks, benefits and preferences.[121,150–153]

Prognostic factors for success influence risk-benefit discussions of immunotherapy. Lower age, such as preschool children may be more successful and allow for completion before significant anxiety.[154] However, lower age will result in many children undergoing OIT, and the risks it entails, who may naturally outgrow the FA.[155] Baseline clinical laboratory analysis repeatedly demonstrates individuals with lower laboratory tests (lower sIgE, lower SPT) have the most success with desensitization,[129] and yet the ones who may stand to benefit most from desensitization may be the ones with the highest numbers. Threshold and severity assessments may also be assisted by biomarkers. The cumulative tolerated dose of allergic reaction was associated with sequential (linear) epitope-specific IgE profiling.[156] The severity of allergic reactions of children was shown to inversely associate with platelet activating factor acetylhydrolase, a stable enzyme that plays a central role in degrading the lipid mediator platelet activating factor.[157] The management of FA has an ongoing need for refinement of patient selection, dose, regimen, and duration. Options to improve immunotherapy must integrate cellular, humoral, and functional biomarkers with clinical history to further understand treatment response.[158] Paramount markers were defined and linked to clinical outcome. (Figure 5)

In summary, the clinical management of FA continues to evolve. The recognized need for long-term treatment will continue to spur fewer intensive approaches to desensitization including fixed dosing, low-dose maintenance targets, alternative routes, and biological adjuvants or monotherapy. Improved ability to risk-phenotype and a patient-centered lens will continue to refine immunotherapy and drive alternative approaches.

Conclusion

Further refinement of potential biomarkers for immunotherapy will contribute to the progressing FA clinical management and facilitate implementation as a clinical routine. Continuous research in rodent models exploring the microbiome and its metabolites' roles can help elucidate their functional effects and novel immune cell subsets. Ongoing trials for FA will reveal additional insights into how best to modify therapies and enhance the safety profile of current treatment strategies.

Conflicts of interest

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Table 1: Main food allergy immunotherapy biomarkers reported in humans [159]

Biomarkers	Mechanism	Readout	Successful OIT	Successful SLIT	Successful EPIT	References
In vivo	**In vivo**	**In vivo**	**In vivo**	**In vivo**	**In vivo**	**In vivo**
Skin Prick Test (SPT)	*In situ* mast cell degranulation	Wheal size	Reduction during therapy	Reduction during therapy	No significant changes	[107,140,160–166]
Oral Food Challenge (OFC)	*In situ* diagnostic test whereby a specific amount of allergen is ingested in a standardized setting to note threshold of responsiveness and tolerance.	Clinical reactivity upon exposure	Increase in threshold of tolerated food	Increase in threshold of tolerated food	Increase in threshold of tolerated food	[96,162,167,168]
Cellular	**Cellular**	**Cellular**	**Cellular**	**Cellular**	**Cellular**	**Cellular**
Basophil Activation Test (BAT)	Measures FceRI cross-linking and basophil activation both by IgE dependent and independent activation by *in vitro* exposure of basophils with extracts or allergens. May also use a passive sensitization strategy involving basophils from healthy donors and serum from allergic individuals.	%CD63+ positivity, CD 203c expression Diamine Oxidase (DAO)	Reduction of %CD63+ during therapy	Reduction of %CD63+ during therapy	Transient reduction %CD63+ during therapy	[15,127,162,163,169–17

Biomarkers	Mechanism	Readout	Successful OIT	Successful SLIT	Successful EPIT	References
Mast cell Activation Test (MAT)	Mast cells are activated through IgE crosslinking of FceRI expressed on cell surfaces. Measures mast cell activation through *in vitro* exposure of mast cells with extracts or allergens. LAD2 or hMCs (Expressing CD117+) May also use a passive sensitization strategy involving mast cells from healthy donors and serum from allergic individuals.	%CD63 and CD107+ positivity	N/A	N/A	N/A	[173–175] *Indirect evidence used to diagnose IgE-mediated FA.
Tregs	Via IL-10 and TGF- ß release (regulatory cytokines), directly inhibiting mast cell degranulation and use CTLA-4 and PD-1 mechanisms [72]	$CD4^+$ $CD25^+FOXP3^+$ in whole blood and isolated PBMCs	Increased during therapy	No significant changes	Induction of LAP1 Tregs *indirect evidence in mice	[112,176,177]

Biomarkers	Mechanism	Readout	Successful OIT	Successful SLIT	Successful EPIT	References
Th2a	APCs present allergen to naïve T cells which provoke differentiation into Th2 responses (Th2a).[178] Th2a increase sIgE, causing IL-4 to prime naïve CD4+ T cells to differentiate into Th2.	CD161, CD49d, classical Th2-related surface markers in whole blood and isolated PBMCs	Reduction during therapy	N/A	N/A	[70,179–181]
DCregs	DCregs produce IL-12, IL-27 and IL-10 skewing immune responses from Th2 to Th1.[178] Downregulation of DC2 cell subset and an increase of DCregs support differentiation of Tregs and Bregs.[182]	C1QA and FCGR3A in whole blood and isolated PBMCs	N/A	Increased during therapy	N/A	[183,184]

Biomarkers	Mechanism	Readout	Successful OIT	Successful SLIT	Successful EPIT	References
Bregs	Br1 cells upregulate IgG4 during their transition to plasma cells.[185] Treg induction via IL-10 and TGF- ß secretion, direct suppression (IL-10) of Teff and indirect (IL-10) via DC inhibition.[186]	IL-10 expression and secretion	Increased during therapy	N/A	Increased during treatment	[185,187] *Indirect evidence.
ILC Type 2 cytokines	ILC2s produce type 2 cytokines (IL-4, IL-5, IL-9, IL-13). Common alarmins recruit ILC2s to release proinflammatory cytokines. Antagonization of Th2 responses because of successful AIT.	N/A IL-4, IL-5, IL-9, IL-13 in whole blood and isolated PBMCs Il-33	N/A Reduction during therapy	Reduction during therapy Reduction during therapy	N/A Tendency to decrease, however not significant during therapy	[65,188] [163,176,189]

Biomarkers	Mechanism	Readout	Successful OIT	Successful SLIT	Successful EPIT	References
Tfh13 cells	Subset of Th cell found in B-cell follicles that controls antibody isotypes switching, affinity maturation and B-cell memory.	CXCR5, PD-1, Bcl-6, Il-21	N/A	N/A	N/A	[190] *Continuous allergen exposure blocks CXCR5 expression in memory Tfh cells.
Antibody based	**Antibody based**	**Antibody based**	**Antibody based**	**Antibody based**	**Antibody based**	**Antibody based**
Allergen and specific IgE (sIgE)	Culprit molecule of IgE mediated allergy. Binds to high-affinity IgE receptor FceRI on surfaces of mast cells, basophils and eosinophils, some sub-types of APCs and via the low affinity IgE receptor FceRII.	Extract-specific IgE Allergen-specific IgE	Transient increase, then decrease throughout therapy	Transient increase, then decrease throughout therapy	Decrease in late phase of therapy	[107,113,191–196,114,1
sIgG4	Soluble IgG4 directly interacts with allergens, membrane-bound IgG4 interferes with allergen-mediated IgE crosslinking and inhibits mast cells activation and basophil degranulation.	Extract-specific IgG4 Allergen-specific IgG4	Increase during therapy	Increase during therapy	Increase during therapy	[113,127,191,197,198,1

Biomarkers	Mechanism	Readout	Successful OIT	Successful SLIT	Successful EPIT	References
sIgA	Tregs (Tr1) secrete IL-10 and TGF-ß to induce class-switch and promote IgG4, IgA production.[104] Similar to IgG4, IgA may act specifically at the level of mucosal surfaces.	Extract-specific IgG4 Allergen-specific IgG4	Increase during treatment	Increased during treatment	N/A	[108,194,199,200]

N/A: Not available

Figure Legends

Figure 1: Pathophysiology of IgE-mediated food allergy

The interplay between the environment and barrier dysfunction drives Th2-type allergic responses. Epithelial cells in the skin and gastrointestinal tract are actively involved in immune responses by producing and secreting cytokines. Alarmins or damage-associated molecular patterns (DAMPs) including IL-33, IL-25, TSLP and PGE2 are epithelial cell-derived cytokines that are central regulators of allergic responses. In addition to the classical effector cells (mast cells, basophils, eosinophils, ILC2 cells, B cells and Th2 cells), other T cell subsets are proposed players in IgE-mediated allergy including: Tfh13, Th22, Th9, Th17, and Tfh2 cells. Immunosuppressive cell subsets including T regulatory cells and the recently described ILCregs also play a role by regulating responses.

Figure 2: Humoral factors influencing the allergic response

The anti-allergenic environment is characterized by a high IgG/IgE ratio and allergen-specific IgG4-mediated inhibition. The pro-allergenic environment includes high-affinity IgE driven by Tfh13 cells, high epitope diversity and proximity, and increased glycosylation content in the constant region of IgE. Low-affinity IgE is not a determining factor for a pro nor anti-allergenic environment.

Figure 3: Immunologic changes during food immunotherapy

Allergen immunotherapy (AIT) is a long-term process. At baseline, individuals with food allergies have effector cells that are reactive to allergens, mounting an allergic response upon above-threshold exposure. During the early phase (days to weeks) of AIT, the threshold of effector cell activation (basophil) increases, resulting in desensitization. Continuation of controlled allergen exposure, result in the generation of regulatory cells that produce tolerogenic IL-10, as well as the increased production of allergen specific IgG4 and IgA with slow reduction of allergen-specific IgE (months-years). Treatment response can be monitored by immunotherapy biomarkers including reduction in skin prick test (SPT) and basophil activation test (BAT) results as well as changes in immune cell frequency and humoral response.

Figure 4: Factors affecting AIT safety and eligibility

The decision to pursue AIT must be patient-centered. Individual patient specific factors can influence the risks and potential benefits of treatment. Medical history and compliance factors may impact the

safety profile of AIT. Administration protocol and psychosocial factors including socioeconomic status are important considerations when stratifying risk and safety.

Figure 5: Diagnosis and monitoring of food allergy and immunotherapy

Integrating cellular, humoral, and functional biomarkers of food allergy with clinical history will help generate tailored, patient-centred treatment options. Identifying and characterizing endotypes to better understand an individual's food allergy pathophysiology will assist in selecting potentially effective treatments and predicting treatment responses.

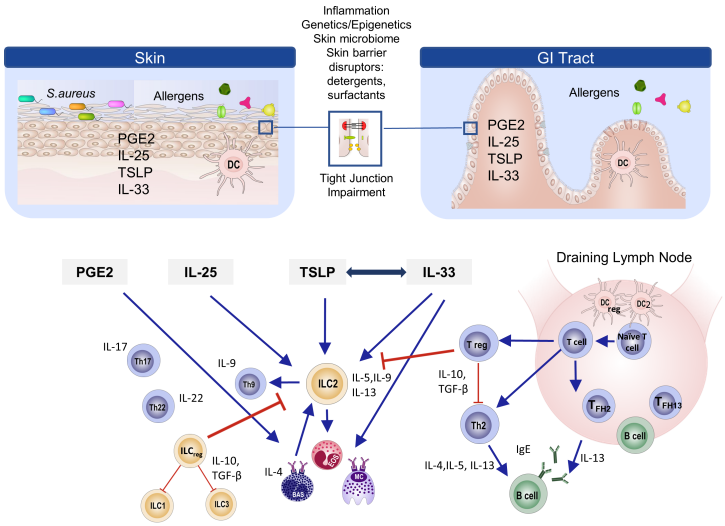
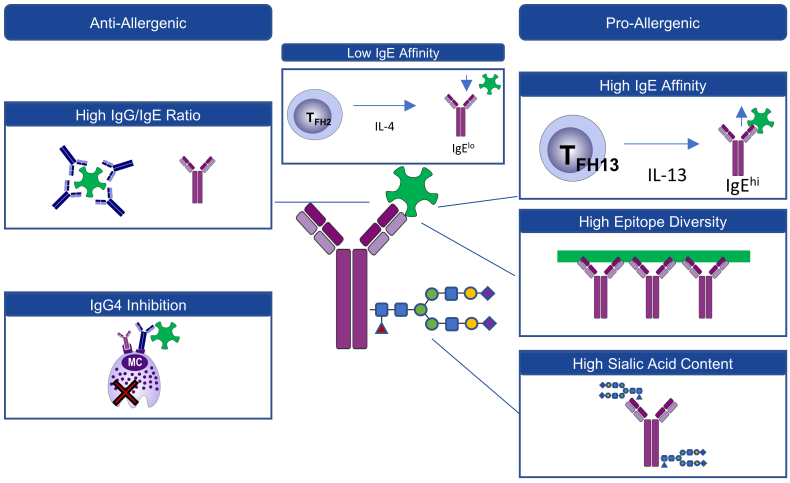


Figure 1_Locke and Hung et al.



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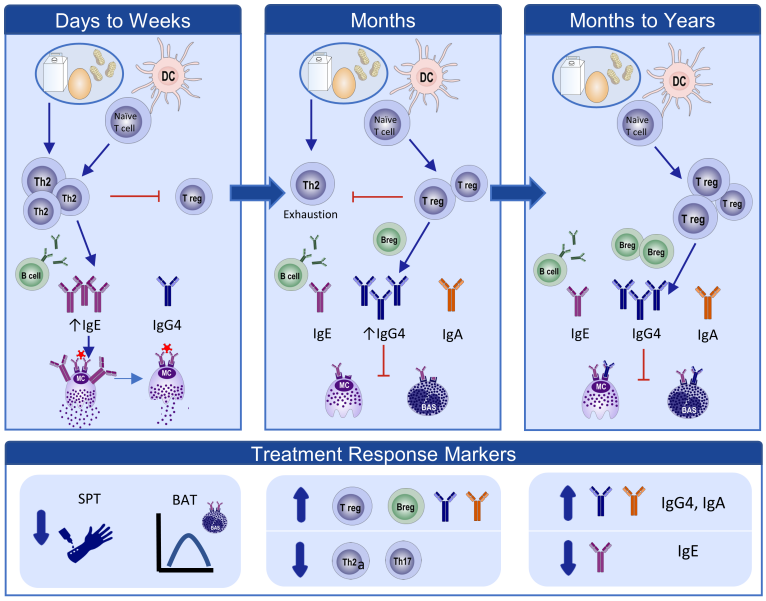

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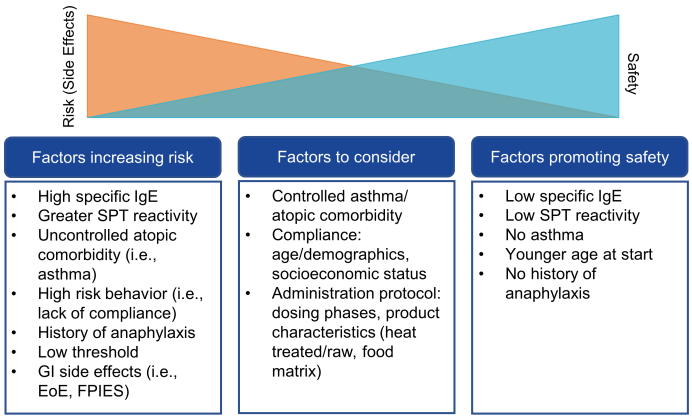

Figure 4_Locke and Hung et al.

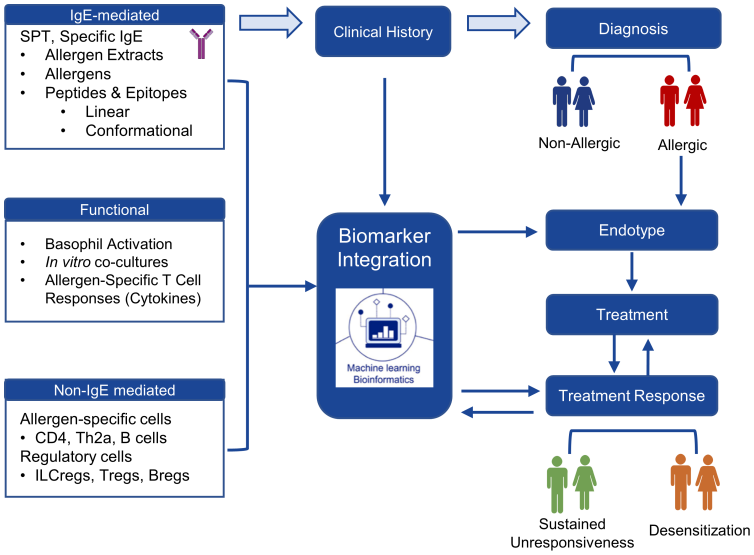

Figure 5_Locke and Hung et al.