



Biomarkers in Food Allergy Immunotherapy

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Abstract

Purpose of Review Investigational allergen immunotherapies (AITs) including oral immunotherapy (OIT), sublingual immunotherapy (SLIT), and epicutaneous immunotherapy (EPIT) have proven to increase allergen thresholds required to elicit an allergic reaction in a majority of subjects. However, these studies lack consistent biomarkers to predict therapy outcomes. Here, we will review biomarkers that are currently being investigated for AIT.

Recent Findings The mechanisms underlying the therapeutic benefit of AIT involve various cell types, including mast cells, basophils, T cells, and B cells. Skin prick and basophil activation tests assess effector cell sensitivity to allergen and are decreased in subjects on AIT. Allergen-specific IgE increases initially and decreases with continued therapy, while allergen-specific IgG and IgA increase throughout therapy. Allergen-induced regulatory T cells (Tregs) increase throughout therapy and were found to be associated with sustained unresponsiveness after OIT. Subjects on OIT and SLIT have decreased Th2 cytokine production during therapy.

Summary Although trends have been reported, a common limitation of these biomarkers is that none are able to reproducibly predict prognosis during AIT. Further studies are needed to expand the currently available biomarker repertoire to provide personalized approaches to AIT.

Keywords Food allergy · Allergen · Immunotherapy · Biomarkers · Basophils · Tregs · IgG4

Abbreviations

AIT	Allergen immunotherapy
BAT	Basophil activation test
DBPCFC	Double-blind, placebo-controlled food challenge
EPIT	Epicutaneous immunotherapy
MAT	Mast cell activation test
OIT	Oral immunotherapy
SLIT	Sublingual immunotherapy
SPT	Skin prick test

Introduction

IgE-mediated food allergy is a life-threatening disease, impacting over 15 million US citizens. Over a 10-year period, there has been an 18% increase in disease prevalence among US children [1, 2]. In most cases, food allergy presents early in infancy and proceeds through adulthood, with an estimated 10% of US adults affected [3]. Currently, no curative treatments for food allergies exist, subjecting allergic patients to maintain a strict avoidance diet, which leads to a decreased quality of life [4]. The eight most common foods to cause a reaction are peanut, tree nut, milk, egg, fish, soy, wheat, and shellfish, with peanut, milk, and egg being the most common among children under the age of 5 years [2].

Allergic immune responses result from dysregulated tolerance mechanisms in the allergic host [5]. The two phases of food allergy are the sensitization phase and the effector phase. During the sensitization phase, food protein is captured and processed by resident dendritic cells in the gut, skin, or airway, then presented on surface MHC class II to naïve CD4 helper T cells. Presentation of allergen to naïve CD4 T cells leads to differentiation into Th2 cells, which secrete high levels of Th2-type cytokines (IL-4, IL-5, and IL-13). IL-4 and IL-13 drive B cells to class switch and differentiate into IgE-producing plasma cells.

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These plasma cells produce allergen-specific IgE (sIgE) that binds to the high-affinity FcεR1s on the surface of mast cells and basophils [5–7]. During the effector phase of allergy, re-exposure to allergen results in crosslinking of FcεR1s leading to degranulation of mast cells and basophils, resulting in the release of pro-inflammatory mediators such as histamine and prostaglandins. These mediators result in the clinical manifestation of allergy commonly seen as urticaria, respiratory distress, GI symptoms, hypotension, etc. [6, 7]

Emerging allergen immunotherapies (AITs) include oral immunotherapy (OIT), sublingual immunotherapy (SLIT), and epicutaneous immunotherapy (EPIT) which differ in routes of administration and antigen dose. Collectively, these food AITs expose subjects to increasing amounts of food allergen over a period of time varying from months to years. Several studies have shown OIT to be effective in inducing short-term desensitization (increased allergen threshold during therapy) in the vast majority of subjects and long-term desensitization or sustained unresponsiveness (SU, absence of allergic symptoms upon allergen re-exposure weeks to months after therapy ends) in a small percentage of subjects [8–10, 11•, 12]. SLIT and EPIT have also been shown to effectively induce desensitization in a subset of patients, although long-term outcomes require further investigation [12–17].

Double-blind, placebo-controlled food challenges (DBPCFCs) are considered the gold standard for diagnosing food allergy [18, 19]. However, these DBPCFCs are time-consuming, expensive, and can place food-allergic patients at risk for anaphylactic reactions. To that end, several biomarkers have been tracked during AIT in an attempt to predict desensitization; however, the identification of biomarkers that accurately and reproducibly predict patient outcomes requires further investigation. This review discusses these potential biomarkers and their ability to predict AIT outcomes, and are summarized in Table 1.

Skin Prick Tests

Mast cells are central effector cells in allergic disease, as they produce allergic mediators that are responsible for clinical symptoms of anaphylaxis [7]. Allergen skin prick tests (SPTs) are diagnostic tests designed to investigate the function of allergen-specific IgE on mast cells. SPTs are conducted by administering minute amounts of allergen to the superficial layers of skin [20, 21]. The allergen binds sIgE, thereby crosslinking FcεR1s on mast cells of the sensitized host, resulting in the release of histamine and other allergic mediators which yield sizeable wheals that are measured by diameter. In general, a wheal size ≥ 3 mm is considered a positive SPT [21]. However, in a study of 467 children (median = 3.0 years of age), food-specific SPT wheal diameters ≥ 8 mm, ≥ 7 mm, and ≥ 8 mm to cow's milk, egg, and peanut, respectively,

were highly predictive with systemic responses to each of these allergens. This indicates that many SPTs yield false positives, resulting from sensitization to an allergen without clinical allergy [22]. Because SPTs are useful diagnostics, they may also be beneficial biomarkers in AIT studies. In one peanut OIT study, the median wheal diameter of the OIT group decreased significantly from 7 mm at baseline to 1.75 mm after 1 year of therapy, compared to no significant change in the placebo group [23]. Similar trends of decreased wheal diameter have been observed in other peanut, egg, and milk OIT studies and in peanut, milk, and peach SLIT studies [12, 13, 24–27]. Few studies have been reported regarding EPIT and SPTs, but results from one study demonstrated no significant changes in subjects after 12 months of peanut EPIT [28••]. Although SPTs are associated with desensitization for groups of subjects on active treatment, they are not ideal predictors of individual AIT outcomes.

Basophil Activation Tests

Similar to mast cells, basophils express FcεR1s that can be crosslinked by food allergens, initiating intracellular signaling events that lead to cell activation [7]. Basophil activation tests (BATs) are functional assays that use whole blood to detect the ability of allergens to activate basophils. BAT samples are analyzed by flow cytometry to detect expression of basophil activation markers (CD63, CD203c) after stimulation with allergens. The detection of CD63 on the cell surface reflects cell degranulation, indicating CD63 is a key biomarker for allergic reactions.

Indeed, the presence of CD63 has been shown to correlate with histamine release in rat basophilic leukemia cells and human basophils [29–31]. CD203c is a cell surface marker unique to basophils and mast cells and its expression is detected at low levels on resting cells, but rapidly upregulated during activation [3]. Other basophil activation markers have been described in the literature, such as CD107a which mimics similar expression patterns to CD63, and CD13 and CD164 which are similar to CD203c [33, 34]. BATs can be used to assess changes during immunotherapy, potentially reflecting the desensitized state of the individual. In a peanut SLIT study by Kim et al., BATs conducted on blood samples collected after 12 months of therapy demonstrated a significant decrease in %CD63⁺ basophils after crude peanut extract stimulation [13]. A similar trend of decreased %CD63⁺ basophil activation was observed in blood from peanut-allergic patients undergoing peanut OIT. In the same study by Thyagarajan et al., subjects allergic to both egg and peanut undergoing peanut OIT had decreased %CD63⁺ basophil activation in response to egg stimulation, indicating OIT may not be allergen specific [35]. In a 2019 study of peanut OIT, early decreases in allergen-induced basophil activation were found in subjects that went on to develop SU, suggesting the potential use of BATs to determine AIT prognosis [36••]. Interestingly, in a

Table 1 Current biomarkers for food allergen immunotherapies

Biomarkers	Targets/readouts	OIT	SLIT	EPIT	References
SPTs	Histamine, wheal size	Decreased after therapy. Allergens: peanut, milk, egg	Decreased after therapy. Allergens: peanut, milk, peach	No changes observed. Allergen: peanut	[13, 23, 24, 28••]
BATs	CD63, CD203c	%CD63 expression decreased after therapy. Allergens: peanut, egg	%CD63 expression decreased after therapy. Allergen: peanut	Decreasing trends after therapy; not significant. Allergen: peanut	[13, 28••, 35, 36••]
sIgE	Whole allergen extracts, components, epitopes	Increased within 1–3 months, then decreased throughout therapy. Allergen: peanut, milk, egg	Increased within 1–3 months, then decreased throughout therapy. Allergen: peanut, milk	Increasing trends early on therapy; not significant. Allergen: peanut	[11•, 13, 46, 47, 50, 28]
sIgG4	Whole allergen extracts, components, epitopes	Continuously increased during therapy. Allergens: peanut, milk, egg	Continuously increased during therapy. Allergens: peanut, milk	Continuously increased during therapy. Allergen: peanut	[28••, 47, 49, 50, 55••]
sIgA	Whole allergen extracts, components	Increased during therapy. Allergens: peanut, egg	Increased during therapy. Allergen: peanut	Not reported.	[37, 58, 59]
Th2 cytokines	IL-4, IL-5, and IL-13	Decreased during therapy. Allergens: peanut, milk, egg	Decreased during therapy. Allergen: peanut	Decreasing trends on therapy; not significant. Allergen: peanut	[13, 28••, 65]
Tregs	CD4 ⁺ CD25 ⁺ FOXP3 ⁺	Number and function increased during therapy. Allergen: peanut	No changes observed. Allergen: peanut	Not reported.	[13, 23]

study assessing the efficacy of both SLIT and OIT, basophil activation did not change in subjects that remained on SLIT for a period of 74 weeks; however, subjects who were initially on SLIT then switched to OIT had a significant decrease in basophil activation within 20 weeks [12]. Similar basophil activation trends have also been reported in a peanut EPIT trial [28••]. During AIT, there is suppression of the allergen-specific basophil response; however, more studies are needed to test correlations with patient outcomes.

Immunoglobulin Quantification

B cells are key immune cells involved in the sensitization phase of food allergy, as they respond to allergic stimuli by producing high-affinity allergen-specific IgE needed for rapid systemic responses. B cells produce additional immunoglobulins aside from IgE, such as IgG and IgA, all of which are modified during AIT [7, 37]. In terms of biomarkers, studies have investigated immunoglobulin quantities to whole food extracts, purified allergen components, and epitopes within allergens [38–44]. Total and allergen-specific immunoglobulins are most often quantified by ImmunoCAP, a highly standardized assay similar to an ELISA [45].

IgE

Although IgE is required for allergen sensitization, high levels of IgE do not directly correlate with reaction severity. Several studies have shown allergen-specific IgE (sIgE) increases during the first 3–6 months of AIT and decreases over the course of therapy. For example, median peanut-specific IgE increased 3-fold from 104 to 308 kU/L within 2 months of peanut OIT, but levels after 1 year of therapy were not statistically different from baseline [23]. Similar trends were observed in other OIT and SLIT studies [11•, 13, 46]. A single EPIT study demonstrated an increase in peanut-specific IgE early on in therapy, but this trend was not significant [47]. However, subjects on long-term OIT and SLIT have significantly reduced sIgE compared to baseline [8, 48]. For example, after 60 months of SLIT, median peanut-specific IgE significantly decreased from 83.9 kU/L at baseline to 20 kU/L after completion of the study [14]. Studies have also measured allergen-specific IgE to different food components during the course of AIT. In a peanut OIT study conducted in the USA, specific IgE to Ara h 1, Ara h 2, and Ara h 3 was quantified and shown to follow similar trends to whole peanut-specific IgE [8, 49]. Component-specific IgE measured in peanut SLIT also followed comparable trends to whole peanut-specific IgE [50]. Epitope-specific IgE can be measured with peptide microarrays displaying the entire sequence of an allergen in 10–15 amino acid

chains. In the context of AIT, IgE specific to peanut epitopes have been investigated and the number of IgE-binding epitopes decreased over the course of OIT [49]. In a recent milk OIT study, high-throughput microfluidic assays were used to quantify milk protein binding epitopes by IgE and IgG4 antibodies before and after therapy. Although this study reports a small sample size, epitope repertoire mapping allowed for prediction of SU outcomes on therapy suggesting its use as a potential biomarker [51]. Ratios of sIgE to total IgE have been shown to be more predictive of food challenge outcomes than specific IgE alone [52]. Therefore, this ratio has been investigated as a potential biomarker during therapy and has been reported to be more informative than specific IgE alone [8]. However, these ratios were examined in a small cohort and need to be replicated with an increased sample size. Collectively, changes in IgE are well-documented during the course of therapy, but more work is needed to determine its use as a predictive biomarker of AIT.

IgG4

Allergen-specific IgG4 (sIgG4) acts as a blocking antibody by competing with allergen-specific IgE in binding allergens and inhibiting IgE-allergen complex formation needed for B cell activation [53, 54]. Modulation of allergen-specific IgG4 levels during the course of AIT has been reported to initially increase and then plateau in later months of AIT. The increase in sIgG4 has been shown for several different foods including peanut, milk, and egg during OIT, SLIT, and EPIT [9, 12, 13, 23, 37, 47, 49, 50]. During peanut SLIT, IgG4 to Ara h 1, Ara h 2, and Ara h 3 were also increased [50]. One study has demonstrated that peanut OIT increased the production of sIgG4 and enhanced the IgG4 epitope diversity, which was not observed in control subjects [49]. Studies have demonstrated that elevated production of sIgG4 during the course of AIT does not correlate with clinical outcomes, suggesting it should not be used alone as a predictor of AIT [8, 26]. Since quantities are not valid predictors, functional assays are emerging to assess potential outcomes of AIT. These assays have investigated the role of IgG4, FcγRIIb, and the blocking capacity of post-OIT and post-SLIT plasma on basophil activation [55•, 56, 57].

IgG4/IgE Ratios

Because it has been shown that IgG4 and IgE alone are not sufficient biomarkers in predicting AIT outcomes, many groups have looked at the ratios between these protective and pro-allergic immunoglobulins. As expected, since sIgG4 is increased throughout therapy and sIgE is substantially lowered, the IgG4/IgE ratios increased. Although this ratio has been uniformly demonstrated to increase over the course of therapy, the ratio is not a strong predictor of individual outcomes [8, 13, 28•].

IgG1, IgG2, IgG3

IgG1, IgG2, and IgG3 are the other subtypes of the IgG immunoglobulin family, which have also been quantified during the course of AIT. Similar to IgG4, these immunoglobulins have been shown to increase within the first few months of OIT, and remain elevated for at least the first year [56]. More studies are needed to investigate changes in these immunoglobulins during therapy and the role they play in predicting AIT outcomes.

IgA

IgA is an immunoglobulin commonly found at mucosal surfaces and is critical for immune exclusion. Importantly, OIT and SLIT allergens are administered at mucosal surfaces and thus may induce IgA production. While measuring IgA from mucosal secretions is difficult, we can measure concentrations of IgA, IgA1, and IgA2 from patient sera during AIT [37, 58]. One study investigated changes in these immunoglobulins during egg OIT and found that all three isotypes were increased within a 2-year period. Egg white-, ovalbumin-, and ovomucoid-specific IgA, IgA1, and IgA2 were all elevated with OIT [37]. Interestingly, subjects that developed SU had higher levels of IgA against egg allergens compared to subjects that were transiently desensitized. Peanut-specific IgA was also increased in subjects undergoing SLIT [59]. Further studies may help determine whether serum IgA is a useful biomarker in AIT.

T Cell Assays

Immune tolerance to food antigens is an active process mediated by specialized regulatory T cells, which upon exposure to antigen in a healthy host secrete anti-inflammatory signals to local immune cells and tissues, maintaining immune homeostasis [7]. Mechanisms underlying the failure in immune tolerance to food proteins that are otherwise harmless are still being elucidated, but dysfunctional regulatory T cells are uniformly believed to be at the center of this phenomenon [60–62]. Upon exposure to allergen at the mucosal surface, antigen-presenting cells present the processed allergen to CD4 T cells via MHC class II molecules, which in turn leads to activation and differentiation into Th2 cells. Th2 cells are categorized by their ability to secrete Th2-type cytokines (IL-4, IL-5, IL-9, IL-13) responsible for B cell differentiation and class-switching to IgE-producing plasma cells. In research settings, allergen-specific T cell subsets and products of T cell activation are emerging as biomarkers for predicting AIT outcomes.

Th2 Cytokines

Secreted Th2 cytokines can be detected upon re-stimulation with allergen *in vitro*. Various methods such as co-culturing T cells with dendritic cells, tetramer analysis, intracellular staining, and ELISpot can all be used to investigate the Th2 (IL-4, IL-5, IL-9, IL-13), Th1 (IFN γ), and Treg (IL-10, TGF β) responses during AIT, and have been reviewed previously [63]. In a 2019 OIT study, peanut-induced IL-5, IL-9, and IL-13 were decreased in desensitized subjects, without any shift in IFN γ and IL-10 [64]. However, another study showed that there was a decrease in Th2 cytokines, with a subsequent increase in Th1 [65]. Additional OIT and SLIT studies have confirmed the reduction of Th2 cytokines, although this may be a transient phenomenon, which reverses upon therapy completion [13, 23, 66]. Subjects on peanut EPIT had decreased Th2 cytokines, but this trend was not significant [28•]. Although several studies have reported a decrease during AIT, they do not predict which individuals will successfully reach desensitization or SU.

Regulatory T Cells

As previously mentioned, regulatory T cells (Tregs) are essential in regulating immune tolerance to food antigens in a healthy host. In the context of food allergy, Treg functions are aberrant, while Th2 cells function to secrete allergy-promoting cytokines. Tregs have been investigated in AIT, with the expectation that their function and numbers are altered during AIT. An early study demonstrated that peanut OIT led to a transient increase in CD4⁺ CD25⁺ FOXP3⁺ Tregs, which was later reproduced in another AIT trial [23, 58]. In a peanut SLIT study, no changes were observed in the number of Tregs after therapy [13]. A limitation of these findings is that only the number of peanut-induced Tregs were investigated, whereas function may be more informative. In a separate study, suppression assays were employed to measure function of Tregs from patients after therapy. Suppression by Tregs was significantly increased, and hypomethylation was found at FOXP3 CpG sites within these Tregs. Importantly, function of Tregs and hypomethylation of FOXP3 CpG sites were associated with the ability to develop tolerance following OIT [67]. In a mouse model of EPIT, OVA allergen administered cutaneously led to the homing of LAP⁺FOXP3⁺ Tregs to the gastrointestinal tract. This study demonstrated an association between the skin and gut and implies a potential mechanism of EPIT. Tregs increase in number and suppressive function over time in AIT; however, further studies with larger sample sizes are needed to confirm their use as a predictor in patient outcomes on AIT.

Th2a Cells

Th2a cells are an allergen-specific subclass of memory Th2 cells that are phenotypically and functionally distinct from conventional Th2 cells. These cells are defined as CD4⁺CD45RO⁺CD27⁺CD45RB^{low}CRTH2⁺CD161⁺CD49d⁺, and changes in this population have been reported in a single peanut OIT study thus far [68•]. As therapy continued, the number of allergen-specific Th2a cells in patients that were on therapy decreased, suggesting an association with fewer Th2a cells and desensitization. These findings suggest the use of Th2a as a biomarker for prognosis in AIT; however, more studies are needed to investigate these cells in OIT, SLIT, and EPIT [68•, 69].

Other Potential Biomarkers

Omics Approaches

Recent high throughput technologies have allowed for the large-scale analysis of biological components, including metabolites, proteins, gene expression, and microbiota [70]. In a peanut DBPCFC study assessing allergic reactions, transcriptome analyses were used to identify six key acute-phase and pro-inflammatory genes unique to the peanut-reactive subjects that could be further investigated for mechanistic studies and therapeutic use [71]. In a separate study, mass spectrometry was used to identify unique metabolites in sera collected from children with food allergy, asthma, or both. Unbiased analyses led to the characterization of food-allergic sera having fewer sphingolipids and lysophospholipids compared to non-food allergic sera [72]. In another metabolomics study, mass spectrometry was used to identify overproduction of uric acid during sensitization in a mouse model of peanut allergy [73]. Various studies investigating the microbiome in food allergy have suggested that specific microbiota can affect allergen sensitization. Food allergy mouse models have been used to identify various classes of bacteria such as *Lachnospiraceae*, *Lactobacillaceae*, *Rikenellaceae*, *Porphyromonadaceae*, *Bifidobacterium*, and *Bacteroides* that were unique to allergic populations compared to wild-type controls [74, 75]. Recently, a study demonstrated that germ-free mice colonized with bacteria from healthy, but not cow's milk-allergic infants protected the mice from developing a milk allergy [76]. Transcriptomics was employed in this study to identify a unique signature between the cow's milk-allergic and healthy-colonized mice. Further exploration and characterization of microbiota can be examined in patient stool samples collected during the course of therapy, and may lead to the identification of new biomarkers.

Mast Cell Activation Tests

Mast cell activation tests (MATs), similar to BATs, measure degranulation of allergic effector cells. MATs utilize the immortalized human LAD2 mast cell line that co-express FcεRI and FcγRIIb, and measure mast cell activation by CD63 up-regulation. In one study, LAD2 cells were treated with plasma from peanut-allergic, peanut-sensitized or non-allergic subjects, then stimulated with peanut extract and assessed for CD63 expression [77]. In this study, LAD2 cells sensitized with plasma from allergic subjects and stimulated with peanut led to significantly increased CD63 expression, whereas plasma from non-allergic individuals did not lead to CD63 up-regulation. MATs show promise in diagnosing food allergy, which warrants their potential application in AIT.

Salivary IgA

Changes in allergen-specific IgA of subjects undergoing AIT have also been detected in saliva. In one study, peanut-specific salivary IgA, secretory IgA, and serum IgA significantly increased throughout the course of peanut SLIT [59]. Changes in peanut-specific salivary IgA were associated with desensitization, suggesting a potential use for this biomarker in AIT prognosis. However, this study was limited by its small sample size and should be confirmed in larger studies. Since salivary IgA is informative, we also speculate that allergen-specific IgA in stool samples will provide more insight into mucosal immunoglobulin behavior during AIT.

Conclusion

Systematic trends in effector cells, immunoglobulins, and T cell subsets have been observed in numerous AIT trials, including decreased SPTs and BATs, decreased IgE with increased IgG and IgA, and increased Tregs with decreased Th2 production. However, these biomarkers do not reliably distinguish subjects that have achieved desensitization or SU. Thus, DBPCFCs are required to determine the success of AIT for individual subjects, which are time-consuming, expensive, and can place the patient at risk for allergic reactions. Further studies are needed to evaluate the current array of biomarkers for individual patient outcomes, with continued efforts to incorporate new approaches.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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