

Novel diagnostic techniques and therapeutic strategies for IgE-mediated food allergy

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ABSTRACT

Background: Immunoglobulin E (IgE) mediated food allergy is a potentially life-threatening condition and represents a heavy burden for patients and their families. Identification of the most suitable way for management of each patient has currently become the primary goal for physicians.

Methods: This study reviewed the current literature related to IgE-mediated food allergy.

Results: The use of innovative diagnostic tools, such as allergen-specific IgG4 determination, basophil activation test, and component-resolved diagnostics, is currently available to facilitate a proper diagnosis of food allergy. After several decades of "passive clinical management" of the disease, which was based only on avoidance of the allergenic food and the use of epinephrine in the event of anaphylaxis, there has been a switch to active treatment. The most recent evidence-practice guidelines strongly recommend the use of immunotherapy as an effective therapeutic option, particularly in cases of allergy to cow's milk, egg, or peanut. The use of omalizumab, in association with immunotherapy or alone, has been tested in several studies, and results on its effectiveness seemed to be encouraging. Other biologics, such as dupilumab, reslizumab, mepolizumab, and other anticytokines therapies, are being investigated. Another interesting future treatment strategy could be the use of DNA vaccines.

Conclusion: In recent years, the management of IgE-mediated food allergy has greatly improved. Knowledge of pathogenic mechanisms, understanding of the disease course, and the introduction of novel biomarkers led to more accurate diagnoses along with the active treatment of patients.

(Allergy Asthma Proc 42:124–130, 2021; doi: 10.2500/aap.2021.42.200129)

Immunoglobulin E (IgE) mediated food allergy (FA) is defined as an adverse health effect due to exposure to a given food in which a specific immune IgE response has been demonstrated.^{1,2} An FA represents a heavy burden for patients and their families because it is a potentially life-threatening condition in the event of anaphylaxis. Although mortality from food anaphylaxis is low, with rates that range from 0.03 to 0.3 deaths per million inhabitants per year, this has a profound adverse impact on the quality of life.³ The prevalence of FAs is estimated as being up to 10%, but there is a strong impression that the prevalence of FAs will further increase in the coming years.⁴ Currently, the hallmarks of treatment remain avoidance of allergenic foods and the use of epinephrine in the event of severe reactions; however, numerous barriers exist. An avoidance diet is often difficult and frustrating for patients with a persistent FA. An elimination diet is also burdened by the risk of accidental exposure to the

triggering food.⁵ Studies have also demonstrated that epinephrine is underused during anaphylaxis due to fears of needles and adverse effects of the drug or poor adherence to treatment.^{6,7}

In this article, we provided an updated review of literature that focuses on the introduction of novel diagnostic techniques and the advent of alternative therapeutic approaches. To accomplish this, we performed a comprehensive search of published literature by using the PubMed MEDLINE data base from January 2010 to September 2020. We used the following string of terms: "IgE-mediated food allergy and diagnosis, biomarkers or molecular biology" and "IgE-mediated food allergy and treatment, biologics or vaccines." Full-text English-language articles were screened and included. Emphasis was placed on evidence-based guidelines and all high-quality studies (randomized controlled studies, observational studies, reviews, and meta-analyses).

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The authors have no conflicts of interest to declare pertaining to this article
No external funding sources reported

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FA PHENOTYPES

Chronic diseases are characterized by complex components, including gene-environment interactions, socioeconomic elements, demographic features, and psychological aspects. Knowledge of these complex factors leads to the identification of a phenotypic approach that has been demonstrated to be useful for

the management of IgE-mediated FAs.⁸ Patient demographic and clinical characteristics, the natural history of FAs, the severity of symptoms, food allergen components, and sensitization profiles are important factors involved in the recognition of different FA phenotypes. IgE-mediated FAs may appear in infancy, toddlerhood, childhood, or sometimes, less frequently, in adulthood. Genetic heritage, gene associations, copy number variations, and epigenetic modifications influence the age of onset. For instance, Serine Peptidase Inhibitor Kazal Type 5 (SPINK5) and Forkhead Box P3 (FOXP3) genes, or human leucocyte antigens (HLA)-DR and HLA-DQ gene loci are involved in the early onset of FA.⁹

Gender is another determinant of an FA phenotype. Peters *et al.*,¹⁰ in their Australian HealthNuts cohort of 5276 infants, demonstrated that boys are more susceptible to developing multiple FAs. Female patients seem to be at a higher risk of developing severe peanut-allergic phenotypes and, consequently, anaphylactic reactions.^{11–13} This different allergy development pattern between the genders could be attributed to the role of sex hormones.¹⁴ Although evidence to date is insufficient to support racial disparity in FA, the ethnic background also seems to influence the FA phenotype.¹⁵ The different odds of food sensitization among the races could be related to the interaction between environmental factors, such as living conditions, cultural dietary habits, and genetic influence.⁸

The coexistence of other allergic diseases strongly influences the course of FAs. Summers *et al.*¹⁶ reported that severe asthma was associated with life-threatening bronchospasms induced by allergenic food ingestion and that severe pharyngeal edema was more common in patients with serious allergic rhinitis. These investigators also showed that severe atopic dermatitis was associated with a significant increased risk of loss of consciousness in the event of severe reactions.¹⁶ Studies showed that atopic comorbidities are associated with the most severe FA phenotype and low eliciting dose during the oral food challenge.^{11,12} Furthermore, the presence of other allergic diseases is related to the persistence of an FA, as demonstrated by the high prevalence of asthma and rhinitis in cases of persistent cow's milk or hen's egg FAs.^{17,18} Also, asthma is considered a risk factor for anaphylaxis or failure of oral immunotherapy (OIT) in patients with cow's milk allergy.¹⁹

During the past few years, component-resolved diagnostics (CRD) have acquired a substantial role in the field of allergy. Selected CRD components have the potential to diagnose FAs with high specificity but low sensitivity.²⁰ CRD can also identify the eliciting allergenic molecule and distinguish genuine sensitization from sensitization due to cross-reactivity.²¹ Currently, the diagnostic accuracy of CRD is mainly related to the more severe allergic phenotypes.²⁰ Casein Bos d 8 and

ovomucoid Gal d1 specific IgE (sIgE) levels have been identified as markers of reactivity in patients with cow's milk and hen's egg allergy, respectively.²² Dang *et al.*²³ described the potential role of sensitization to multiple egg allergens (*i.e.*, Gal d 1, Gal d 2, Gal d 3, Gal d 5) as a prognostic marker for long-lasting egg allergy. With regard to peanut allergy, the absence of sIgE to Ara h2 seems to be associated with less severe reactions.²⁴ Furthermore, high sIgE levels to Ara h 1 and Ara h 8 could be associated with the persistence of allergy.^{25,26}

CRD may explain the difference of sensitization profiles from one geographic region to another. For instance, in the United States and western Europe, peanut allergy is linked to a primary sensitization to the 2S albumin allergen r Ara h2 but also r Ara h 1, 3, and 6. However, in northern and eastern Europe, peanut allergy has been demonstrated to be frequently related to sensitization to Ara h 8, which belongs to the protein family PR 10, whereas, in Mediterranean countries, it is secondary to a primary sensitivity to Ara h 9, which is a lipid transfer protein.⁸ Another study reported that peanut allergy was linked to r Ara h 2 sensitization (90%) in the United States, r Ara h 9 (60%) in Spain, and r Ara h 8 (65%) in Sweden.²⁷ Also, undetectable levels of sIgE to Cor a 9 or Cor a 14 may be considered reliable predictors of less-severe clinical reactivity in patients with hazelnut allergy.^{28,29}

The identification of the exact eliciting allergen molecule allowed the outlining of some sensitization profiles that are remarkable due to their pathogenetic and clinical characteristics. The most relevant are the following: birch pollen-related syndrome, caused by Bet v 1 proteins from plant-based foods; galactose- α -1,3-galactose allergy, which is a reaction to a carbohydrate epitope largely found on proteins and lipids in nonprimate mammals; and lipid transfer protein syndrome, characterized by sensitization to homologous proteins.^{30–34}

NOVEL BIOMARKERS IN FA

In the past few years, the efforts of researchers have aimed to identify more specific, sensitive biomarkers for the determination of an accurate diagnosis, reaction severity, prognosis, and evaluation of treatment efficacy. Therefore, *in vitro* tests are increasingly under investigation. The basophil activation test (BAT) uses flow cytometry to assess the expression of activation markers, such as CD63 and CD203c, on the surface of live basophils that are upregulated after the cross-linking of IgE antibodies bound to the high-affinity IgE receptor that results from allergen or anti-IgE stimulation.³⁵ BAT has been shown to be accurate, especially in peanut allergy, and is able to distinguish patients who were clinically allergic from those who were tolerant albeit sensitized.^{36,37} BAT may also be

used to define the severity of allergic reactions because individuals with more severe reactions show a greater proportion of activated basophils.

In subjects who reacted to small amounts of the allergen, basophils start activating at a lower allergen dose, which shows a greater basophil sensitivity.^{38,39} Also, BAT may explore the mechanisms of allergen immunotherapy (AIT) at the basophil level. A reduction in basophil reactivity has been traced not only to the culprit allergen but also to the bystander allergen (e.g., egg) during peanut OIT.⁴⁰ However, sensitivity in predicting the oral food challenge outcome remains low.⁴¹ Further emerging diagnostic tests include the mast cell activation test and histamine-release assays. The mast cell activation test aims to assess the ability of the patient's allergen sIgE antibodies to elicit mast cell degranulation. Histamine-release assays measure the amount of histamine that is released by activated basophils. These novel diagnostic investigations are currently undergoing validation.⁴²

Allergen sIgG4 determination and food specific IgE/IgG4 ratios have increasingly acquired a sustained role in predicting the natural history of FAs and the response to immunotherapy. Ovalbumin and ovomucoids specific IgE/IgG4 ratios are higher in patients with allergy to baked egg.⁴³ Conversely, an increase in the ovomucoid sIgG4/sIgE ratio during immunotherapy was demonstrated to be associated with successful clinical outcomes.⁴⁴ With regard to cow's milk allergy, Caubet *et al.*⁴⁵ showed that the intensity of binding IgG4 antibodies to cow's milk peptides was related to the persistence of milk allergy. A multicenter study found that an increase in the IgG4 concentration to milk components during immunotherapy was related to effective desensitization.⁴⁶ Also, a higher peanut sIgG4/sIgE ratio seems to be related to the decreased frequency of development of peanut allergy in cases of early introduction of peanuts in infants.⁴⁷

Recent research aimed to report the ability of mucosal biomarkers in identifying patients with an FA. A systematic review has recently been conducted to evaluate the role of mucosal biomarkers associated with FAs. This review showed that gut and/or fecal biomarkers, above all eosinophil cationic protein, fecal calprotectin, and α 1-antitrypsin, have emerged as potentially promising candidates in the diagnosis of FA. Other cell types, such as CD103⁺ dendritic cells, C-X-C Motif Chemokine Receptor 1 (CXCR1)+ macrophages, and FOXP3⁺ regulatory T-cells were also involved.⁴⁸

MOLECULAR ALLERGOLOGY

Significant advancements in molecular biology techniques, such as DNA cloning, protein expression and structural analysis, microarray analysis, peptide

synthesis, and next-generation sequencing, have led molecular allergology to be considered as a useful tool in the field of FA. Molecular studies allow obtaining information on the molecular and structural basis of food allergens and provide cutting-edge tools for FA diagnosis and therapy.⁴⁹ High-resolution mass spectrometry is currently one of the most important techniques for the molecular characterization of allergens, including structural modification, degradation in the gastrointestinal environment, or identification of suitable marker peptides for the development of novel analytical approaches.⁵⁰

Numerous studies aimed to assess different epitopes (*i.e.*, regions of the allergen recognized by specific antibodies) in allergenic molecules. Epitopes are generally divided into linear or conformational, depending on whether they correspond to a continuous amino acid sequence or a group of amino acids that are oriented in close proximity within each other in the three-dimensional structure of the folded allergen.⁵¹ The identification of linear epitopes is easier through the execution of immunoscreening assays of allergen-derived overlapping synthetic peptides with the sera of patients with allergy. Instead, the identification of conformational epitopes is based on different approaches, including molecular analysis by combinatorial techniques and by structural and bioinformatic analysis of the allergen molecules.

An exemplificative case is represented by studies conducted on tropomyosin, a major crustacean allergen. Ayuso *et al.*⁵² identified five major IgE-binding regions of the allergenic shrimp tropomyosin Pen a 1 by using immunoreaction assays with the sera from 18 subjects with shrimp allergy. These major epitopes are positioned at regular intervals of ~49 amino acids (7 heptads), which suggests a relationship with the repetitive coiled-coil structure of the tropomyosin molecule.⁵² More recently, eight linear epitopes and seven conformational epitopes of tropomyosin from *Scylla serrata* were identified by using phage display. After the exclusion of sequences that contain trypsin cutting sites, 12 synthetic peptides were synthesized to test their allergenicity against the sera of 10 subjects with crab allergy. Among those peptides, one linear epitope and two conformational epitopes were found to immunoreact with all the patients' sera.⁵³

CURRENT AND FUTURE TREATMENTS

The European Academy of Allergy and Clinical Immunology has developed clinical guidelines that state that OIT can be recommended as a treatment option to increase the threshold of reaction in children with persistent cow's milk, egg, or peanut allergy.⁵⁴ During OIT, a food allergen(s) is combined with a vehicle food and consumed in incrementally increasing

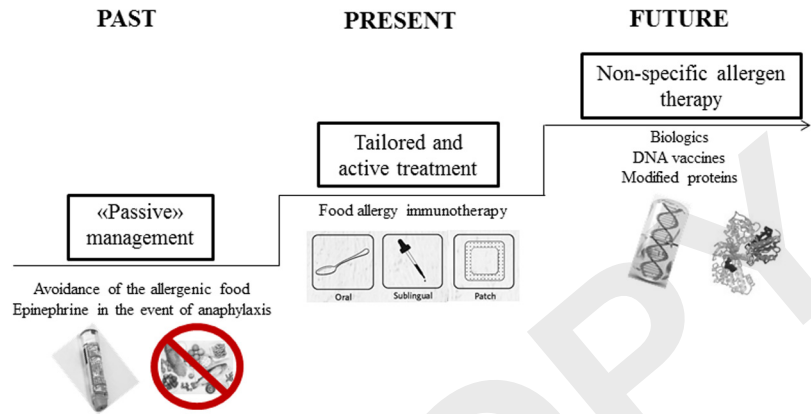


Figure 1. Evolution of the treatment of immunoglobulin E (IgE) mediated food allergy from a passive approach (avoidance and drugs) to active cure (food immunotherapy) and other future developments.

doses over a specified time interval to promote immunomodulatory effects that lead to the clinical outcome of desensitization.⁵⁵ OIT marks a breakthrough module compared with previous thinking that focused on avoiding reactions at all costs; however, it requires a complex, demanding effort on the part of both patients and clinicians.⁵⁶

Other routes of administration of FA AIT include sublingual immunotherapy (SLIT), epicutaneous immunotherapy (EPIT), and subcutaneous immunotherapy (SCIT). SLIT is an approach that involves sublingual delivery of a daily dose of food protein in a liquid concentrate that is held in place for a couple of minutes, then spit out or swallowed. The SLIT dosage is lower than the OIT dosage and is limited to the concentration of available extracts, which thus reduces the risk of adverse reactions but at the expense of reduced efficacy.⁵⁵ With EPIT, an allergen-containing patch is applied to the skin to facilitate the absorption of the food allergen by dendritic cells in the dermis that presents the antigen and promotes the generation of regulatory T-cells.⁵⁷ Recent clinical trials demonstrated that EPIT for peanut allergy was effective and safe beyond long-term follow-up.^{58,59} More data are awaited to better understand the response to EPIT, including the time to achieve response and floor-ceiling effects relative to gain from an entry eliciting dose.⁶⁰ Also, SCIT is characterized by subcutaneous allergen administration in incrementally increasing doses. Preclinical SCIT trials use an alum-adsorbed hypoallergen that has been modified chemically or with site-directed mutagenesis to reduce IgE-binding capacity.⁶¹

Nonspecific allergen therapies, such as biologics, probiotics, modified proteins, and DNA vaccines, are in the early phase of development and, depending on results, could also become important treatment options.⁶² Some studies^{63–65} were conducted to evaluate the role of omalizumab (anti-IgE monoclonal antibody), in association with OIT or alone, as a possible therapeutic option. Results from these studies

encourage the safety and effectiveness of omalizumab as a therapeutic weapon to increase the threshold reaction to the allergenic food, which provides more protection in cases of accidental ingestion.^{63–65} To avoid repeated anti-IgE administrations, gene therapy by using adenovirus vectors that code for omalizumab was proposed and tested in a humanized murine model of peanut allergy. Analysis of data showed that a single administration protected the humanized murine model from FAs.⁶⁶ However, further studies are awaited to better investigate the efficacy and safety of gene therapy, and its application in humans, particularly with regard to the impact of such treatment on quality of life. Other biologics, such as dupilumab (anti-interleukin-4R α), reslizumab (anti-interleukin-5), mepolizumab (anti-interleukin-5), etokimab (anti-interleukin-33), along with anti-cytokines therapies are also under investigations^{67,68} (Fig. 1).

By following the concept that colonization of the gastrointestinal tract with health-promoting organisms leads to positive benefits, numerous studies have been designed to evaluate the use of probiotics in the prevention and treatment of FAs. According to some investigators, the administration of probiotics, through modulation of the host-gut ecosystem and, consequently, the local metabolism, could allow achieving a tolerance-associated microbial profile.⁶⁹ A 4-year-follow-up study demonstrated that the use of *Lactobacillus rhamnosus* as an adjuvant to OIT led to sustained tolerance in patients with peanut allergy. These investigators showed that the co-administration of *Lactobacillus rhamnosus* and peanuts leads to reduced serum sIgE levels, along with wheals in peanut skin-prick tests, and increased serum sIgG4/sIgE ratios.⁷⁰ However, the main weakness of this study was that probiotic plus OIT treatment was compared with probiotic plus placebo rather than to OIT plus placebo. Hence, it was not possible to delineate the contribution of the preventive and therapeutic effects of probiotics associated with OIT.

Another approach to improve the safety of FA AIT is related to the use of recombinant allergens that have been altered to still induce desensitization along with a

lower risk of IgE-mediated reactions. Zuidmeer-Jonghejan *et al.*⁷¹ tested an *Escherichia coli* produced mutant Cyp c 1 (fish) purified by using standard chromatographic techniques to generate an alum-adsorbed, hypoallergenic carp parvalbumin vaccine for SCIT in patients with fish allergy. This vaccine was approved due to its hypoallergenicity with retained immunogenicity, and clinical trials are ongoing. Alternative therapies with HAL-MPE1 (a chemically modified aluminum hydroxide-adsorbed extract for subcutaneous administration), and PVX108, which is a product composed of synthetic peptides that represents T-cell epitope sequences from Ara h1 and Ara h2 (peanut), are also under investigation.^{72,73}

Another future treatment for FA could be the use of a DNA vaccine. Researchers have designed a DNA vaccine encoding CryJ2 allergen from the Japanese red cedar (JRC) pollen and lysosomal associated membrane protein 1 (LAMP-1) to treat JRC allergy. Analysis of data collected in a phase I trial suggested that the CryJ2-LAMP DNA vaccine is safe and may be immunologically effective in treating JRC-induced allergy.⁷⁴ Recently, the efficacy of MEM49- and MED171-based DNA vaccines (pMEM49 and pMED171) in modulating shrimp allergy in a murine model of shrimp tropomyosin sensitivity have been evaluated.⁷⁵ The effectiveness of hypoallergenic-encoding DNA vaccines in reducing allergenic activity *via* the vigorous induction of functional regulatory T-cells has been demonstrated, and clinical trials in human subjects are now awaited.

CONCLUSION

In the past, the management of FA was generic. As the pathogenetic mechanisms of FA became clearer, new perspectives emerged for both management and treatment, mainly in recent years. The knowledge of FA phenotypes and the detection of biomarkers support a more accurate diagnosis, along with the active treatment of patients. In this context, FA AIT represents the new therapeutic approach for IgE-mediated FA. However, it can be practiced only in selected medical centers and under strict medical supervision. Therefore, in the future, an attempt to encourage the development of more innovative diagnostic tools, along with therapeutic strategies to implement evidence practice guidelines, is needed.

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