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IgE – the main player of food allergy

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Food allergy is a growing problem worldwide, presently affecting 2–4% of adults and 5–8% of young children. IgE is a key player in food allergy. Consequently huge efforts have been made to develop tests to detect either the presence of IgE molecules, their allergen binding sites or their functionality, in order to provide information regarding the patient's food allergy. The ultimate goal is to develop tools that are capable of discriminating between asymptomatic sensitization and a clinically relevant food allergy, and between different allergic phenotypes in an accurate and trustworthy manner. This may generate better diagnostic, prognostic and therapeutic monitoring tools for the future.

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Introduction

Immunoglobulin E (IgE)-mediated food allergy is an immunologic, non-toxic adverse reaction to otherwise harmless antigens in food. The mechanisms underlying IgE-mediated food allergy consist of a sensitization and an elicitation phase (Fig. 1). Sensitization may occur upon the first contact with the food allergen, and results in generation of allergen-specific IgE (sIgE). Elicitation of symptoms occurs upon subsequent contact with the respective allergen leading to symptoms. Symptoms occur within minutes to hours of allergen ingestion [1], and involve one or more of the following systems; the skin (pruritus, urticaria, or angioedema), the gastro-intestinal tract (diarrhea, vomiting, contractions, increased bowel movement), the respiratory tract (asthma attack, hoarseness, stridor/laryngeal angioedema) or the cardiovascular system (dizziness, drop in blood pressure, loss of consciousness) [2,3].

Food allergy appears to be a rising problem worldwide, and currently affects 2–4% of adults and 5–8% of young children [4,5].

Although there is some evidence that the first year of life is decisive to develop allergies or asthma later on, the time point an allergic sensitization occurs is very individual. Despite of crude patterns of sensitization (food allergy in early childhood vs sensitization to inhalant allergens later on), sensitization may already occur *in utero* or at any time point after birth [6]. Most likely a combination of genetic predisposition, pro-allergenic, environmental factors and allergen exposure is required to induce sensitization and overcome natural mechanisms of tolerance induction to innocuous environmental antigens. Mechanisms that are responsible for tolerance maintenance in sensitized individuals and for the re-induction of tolerance in allergic individuals are not

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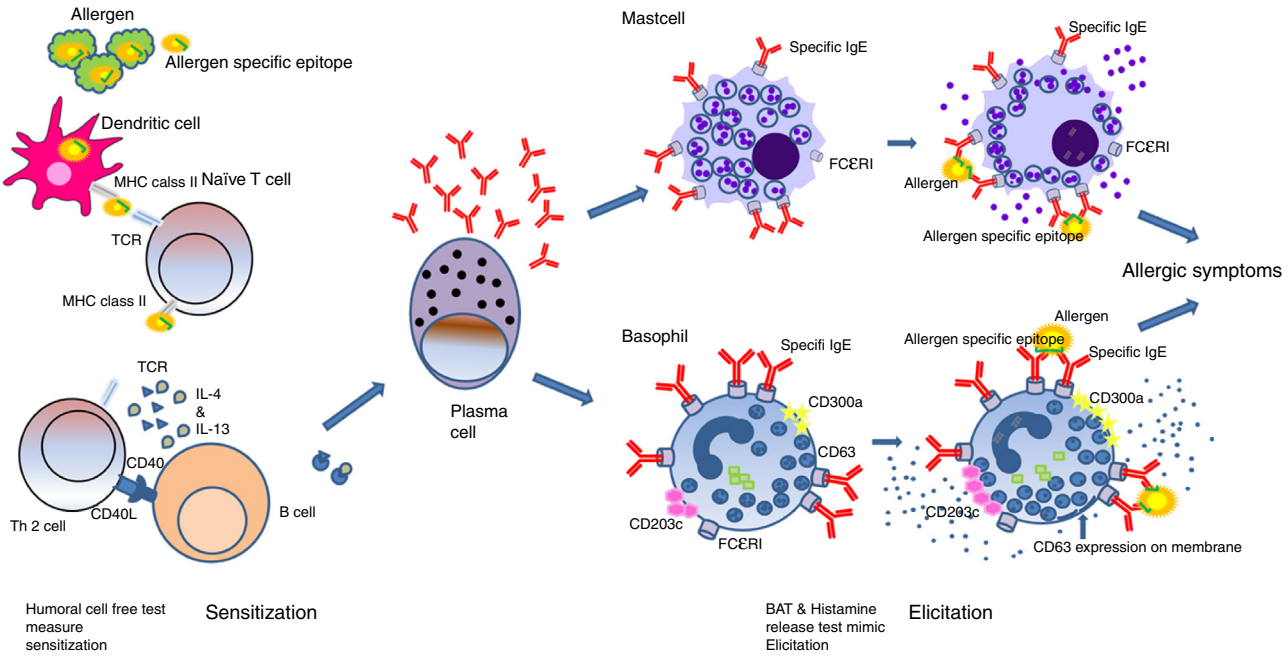

Figure 1. The role of IgE in the context of IgE-mediated allergy. The pathogenesis of IgE-mediated food allergy is divided into two phases; a sensitization phase and an elicitation phase. In the sensitization phase the allergen is taken up by antigen-presenting cells, leading to activation of Th2 cells, which again contributes to the activation of B-cells that differentiate and proliferate into IgE secreting plasma cells. This forms the basis for the cell-free IgE-based *in vitro* test methods as well as the IgE epitope mapping based tests, based on IgE from the food allergic patients. In the elicitation phase allergens may, upon reexposure, cross-link FcεRI bound IgE on mast cells and basophils leading to mediator release and the symptoms characteristic of food allergy. This forms the basis for the cell-based *in vitro* test methods, based on the functionality of the sIgE repertoire rather than just the presence of sIgE.

well understood. Proposed mechanisms include regulatory T-cells, blocking antibodies, tolerogenic dentritic cell populations, lack of epitope diversity and clonal deletion due to constant exposure [6].

The gold-standard for food allergy diagnosis is the oral food challenge (OFC), but it is expensive, time-consuming and carries a risk of severe reactions [4,5]. Hence, there is great interest in developing diagnostic *in vitro* methods. After the discovery of IgE, allergen-sIgE-based tests were developed for diagnosis and have resulted in the standard we use today. Despite of good clinical applicability, limitations of these tests have led to considerable efforts in investigating the role and clinical value of IgE binding to specific allergens as well as IgE binding to specific sites on the allergen. Detecting sIgE binding patterns could be a promising approach to predict food allergy and the associated clinical manifestations [7]. This review discusses the applicability and value of IgE, its binding specificity and functionality in the context of food allergy, in order to predict patient's individual clinical history and to assess treatment efficacy.

IgE based approaches

Immunoglobulins, also designated antibodies, are produced by B cells and consist of two heavy and two light chains. The Fc-region (consisting of the heavy chains) of IgE binds through the high affinity Fc-receptor (FcεRI) to other cells of the immune system, while the Fab region (part heavy and variable light chains) binds to the antigen [8,9]. The binding site of the Fab region (the paratope) binds to a specific part of the antigen, in case of allergy an allergen, which is called the epitope. When an allergen cross-links two FcεRI-bound IgE antibodies on either mast cells or basophils, these effector cells degranulate and release mediators such as histamine, prostaglandins, and leukotrienes, causing the allergic symptoms of food allergy [1].

Various IgE-based tests have been developed in order to provide information about food allergy. These methods can either be cell-free or cell-based (Table 1).

Cell-free IgE-based in vitro *test methods*

Total IgE

Total IgE can be measured by multiple methods and is measured in international units (IU)/mL. Competitive displacement radioimmunoassay (RIA), two-sided immunoradiometric assays (IRMA), two-sided enzyme immunoassay (EIA), and kinetic nephelometry are the currently favoured methods [10].

The clinical applicability of total IgE is limited. IgE is not necessarily specific to food allergens and can be elevated in other atopic diseases, infections and primary immunodeficiencies. Additionally, a low total IgE does not exclude a food

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Table 1. Pros and cons of methods used for detection of sIgE and its functionality.

Humoral and cell based IgE test	Pros	Cons	References
Total IgE	Easy method	Limited clinical applicability	[10,11]
Specific IgE RAST/FEIA	Performed in both commercial and research laboratories Relatively quick assay Levels positively correlate with likelihood of clinical allergy for many foods	Need for clinical validation False negative and false positive results may occur Results not interchangeable with other sIgE tests Recent approved CAP assay has better quality	[12,13]
Specific IgE ELISA	Performed in both commercial and research laboratories	Need for clinical validation False negative and false positive results may occur	[12,13]
		Results not interchangeable with other sIgE tests	
Specific IgE Immunoblot	Allows for identification of both linear and conformational peptides Specific protein recognition	Laborious method	[12,13]
Components ISAC microarray	Easy method Large data output Able to distinguish a clinical relevant allergy and severity for some foods	High cost Not sensitive for all foods Not available or applicable for all foods	[19,22–24]
Basophil Activation Test	Highly specific and sensitive for several foods	Laborious method False negative results may occur No established extracts Not enough clinical data available Not suitable for screening approaches	[36–40]
Humanized RBL	Easily standardized	Laborious method Need for validation No established extracts Low stability	[42,43]
Histamine release assay	Mimics mast cell activation at a larger scale	Laborious method High cost	[46,47]

allergy. An expert panel has advised against using total IgE in diagnosing food allergy [11].

Specific IgE

Allergen specific IgE (sIgE) can be measured by multiple methods and is measured in units of allergen (UA)/mL. Enzyme-linked immunosorbent assay (ELISA), enzyme allergosorbent test (EAST), fluorescence enzyme immunoassay (FEIA), radioallergosorbent test (RAST) and immunoblotting are methods currently applied for measurement of sIgE [12]. Measurement of sIgE typically involves using allergens bound to a solid phase to capture IgE and is quantified by the use of labeled anti-IgE antibodies. These tests are performed both by commercial and research laboratories as well as in many hospital settings. The sIgE levels obtained for a particular protein by different commercial tests are not inter-changeable. There are no international standards for specific IgE assays but rather they are calibrated with the WHO reference preparation for total serum IgE [10].

sIgE levels usually positively correlate with the likelihood of having a clinically relevant food allergy – the higher the sIgE to a given food, the higher the likelihood of clinical reactions upon ingestion. However, the ability to rule out allergy (sensitivity, percentage of allergic individuals with a negative test) and to diagnose allergy (specificity, percentage of individuals with positive test that are allergic) is limited and there is significant variability across populations [13]. sIgE to the respective food may be observed in subjects without a clinical relevant food allergy and it may not be detected in those with a confirmed food allergy [14,15]. This is illustrated by a population-based birth cohort study from the UK where 12 percent of children were sensitized to peanut, but only 2 percent were peanut allergic [16]. Both, the indication to perform sIgE measurement and the assessment of the clinical relevance of a given sIgE value require individual assessment by a clinician. Neither the test nor the interpretation should be done without knowledge of the patient's history.

The sIgE/total IgE ratio has been examined regarding additional diagnostic utility with mixed results. It has been reported that it did not contribute to the diagnosis beyond the sIgE [17]. However, a recent study suggested superiority of

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the sIgE/total IgE ratio as compared to sIgE alone to appropriately assign patients to a food challenge [18]. Overall the clinical applicability of this ratio requires more data before being integrated into clinical decision making.

Component resolved diagnosis

As clinically available test methods utilizing sIgE binding *in vitro* are generally not as efficient as a food challenge in diagnosing food allergy, attempts to further improve the diagnostic accuracy of sIgE testing have been made by introducing the terminus component resolved diagnostics (CRD). It defines reactivity to individual allergens and not to an allergen extract. The binding patterns to homologous allergens from different species may be explained by cross reactivity amongst proteins within the same protein family [19].

For some food allergies the usage of component resolved diagnosis has been proven to increase the ability to predict clinical reactivity [20,21]. This is reflected in superior receiver operator curves integrating sensitivity (proportion of allergic patients correctly identified) and specificity (proportion of non-allergic individuals identified) as compared to extract testing. Currently there is good evidence for the usage of CRD in diagnosing peanut and hazelnut allergy. Further, detection of IgE against specific allergens within these foods, such as the peanut allergen Ara h 2 and the hazelnut allergens Cor a 8, Cor a 9 and Cor a 14, have been shown to predict a clinically relevant food allergy, as well as to help in distinguishing between cross-reactive and 'true' sensitizing allergens [22–24].

CRD has improved our knowledge on the sensitization patterns of some of the more prevalent allergen sources, including peanut, tree nut, egg and milk, but also some less prevalent allergies such as wheat dependent exercise induced allergy and soy allergy [25,26]. In addition to its diagnostic values, CRD may confer therapeutic importance for the development of allergen-specific immunotherapy, as it may enable us to use only the clinically relevant allergens [27].

IgE avidity/affinity

Affinity (the attractive force between substances or particles that causes them to enter into and remaining a chemical combination) of an antibody for its antigen has been shown to be an important determinant of the biological efficacy of the antibody [28]. Measuring the affinity of a single clone of IgE antibodies or the avidity (the additive strength of multiple affinities of non-covalent binding interactions) of a polyclonal IgE antibody response in serum is difficult because of the low serum concentrations of IgE (~150 ng/mL [29]), and sIgE levels are only a fraction of the total serum IgE. In contrast to vaccine research, affinity and avidity measurements to allergens are not commonly used. Nevertheless, El-Khouly *et al.* [30] showed in a study investigating the antibody avidity characteristics of peanut allergic patients that the peanut allergen Ara h 2-specific avidity correlated with the severity as measured by a food challenge score. Shortly afterwards, Wang *et al.* [31] reported that IgE affinity correlated with severity of milk allergy. Recently, Surface Plasmon Resonance imaging, has led to satisfactory measures of the affinity of human IgE antibodies [32]. Despite only being scarcely described affinity/avidity measures could be a promising future tool providing information on the food allergic disease.

In vitro *functional assays*

Various cell-based methods for an indirect analysis of the performance of sIgE have been developed using surrogate biomarkers of effector cell activation such as surface markers or released mediators [33]. Mediators which have been investigated include histamine, heparin, tryptase, chymase, carboxypeptidase A3, prostaglandin D2 and cysteinyl leukotrienes. However, none of these biomarkers have yet proven to be of more value than existing allergy tests [33].

Basophil activation test

Human basophils can be stimulated with allergens *in vitro* and the ability to activate them can be linked to food allergy. In the basophil activation test (BAT), activation of basophils *via* allergens is reflected in an up-regulation of the cell-surface molecules CD63 or CD203c [34]. BATs have been used in the diagnosis, management and as a tool to decide the performance of OFC in milk, egg and peanut allergy, and also in the diagnosis of pollen food syndromes, as reviewed elsewhere [35]. BAT has in some instances shown higher specificity and negative predictive value than sIgE measurement, without losing sensitivity or positive predictive value [36]. In particular, in young children with peanut allergy the BAT proved to be superior to other diagnostic tests in discriminating between peanut allergy and tolerance and the results are encouraging that BAT may significantly reduce the need for OFCs in the future [37]. In the context of ascertaining degrees of baked milk product tolerance the BAT results reached a statistically significant trend [38]. For discriminating between peanut tolerance and reactivity in adult peanut sensitized individuals [39] the BAT showed some utility. Recently, passive sensitization of basophils with sera from allergic donors after stripping of membrane bound IgE has provided promising results in peanut allergic individuals which await confirmation [40].

Humanized RBL assay

Humanized rat basophilic leukemia (RBL) cell-lines transfected with human FcεRI have been developed for the use in functional allergen–IgE interaction research [41]. Humanized RBL cells can be cultured permanently, providing improved standardization. However, this test has not found widespread acceptance among clinicians [42], likely because of the overall low stability of the humanized RBL assay due to

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loss of the humanized receptor. These assays have a lower degree of sensitivity as compared to human basophil tests. The most recent degranulation assay developed is based on the huFcεRI-RBL-2H3 cells, which was tested for sensitivity and specificity for food allergens [43]. Nevertheless, the non-humanized version of the RBL assay, has shown to be efficient for studying the IgE functionality [44,45].

Histamine release assay

Histamine release *in vitro* by stem cell derived mast cells loaded with serum derived IgE may possess the ability to test food-induced mast cell activation at a larger scale due to recent improvements [46]. However, currently this test is not applicable in a real life diagnostic setting because of its costs and the complexity of the method [47].

IgE epitopes based approaches

IgE binding epitopes, defined as the allergenic regions of the allergen recognized by IgE molecules, are generally categorized as either linear or conformational based on the vicinity of the amino acids in the primary structure being involved in the IgE binding [48]. Whereas the linear epitope consists of a contiguous stretch of amino acids juxtaposed in the primary structure, the conformational epitope consists of amino acids distant from one another in the primary structure but brought together by the structural folding of the protein [49–51].

There is no clear boundary at the amino acid level for those amino acids which comprise the epitope [50,52]. Antibody binding epitopes have been suggested to consist of approximately 15 amino acids [53], but there is no evidence that each amino acid in the epitope necessarily interacts with the antibody, and energy calculations have indicated that as few as five to six amino acids are the actual contributors in the binding between epitope and antibody [50,54,55].

Various methods can be applied for identification of IgE binding epitopes, however, for experimental reasons some approaches only allow for identification of the linear type (Table 2).

Methods for identification of linear epitopes

Several IgE epitope mapping methods are based on binding of IgE molecules to peptides derived from the primary structure of the allergen [56,57], thereby allowing for the identification of only linear epitopes. The epitope mapping technology of such peptide arrays, by means of immobilized peptides on a surface, have been subjected to rapid and substantial development over the last decades [58,50]. Typically, overlapping peptides of 10–20 amino acid residues are synthetized in parallel, for example, on a glass slide or a nitrocellulose membrane [60]. Just a few years ago standard peptide synthesis could only synthetize a few hundred peptides, but with the more recent improvements in the field synthesis of up to 2 100 000 peptides in parallel is now a possibility [61]. These advances in peptide arrays have recently allowed for the identification of epitopes on the amino acid level [62]. By substituting each amino acid in the synthetic peptides with an alanine (alanine scan) Hansen *et al.* [62] were able to identify the amino acids within an epitope contributing to the binding to IgE of peanut allergic patients.

Methods for identification of conformational epitopes

Identification of conformational IgE binding epitopes requires more sophisticated techniques, such as X-ray crystallography, nuclear magnetic resonance (NMR), site-directed mutagenesis or phage display technology [50,56,60]. The only complete method for identification of an IgE binding epitope is co-crystallization of an allergen:IgE antibody complex by X-ray crystallography [56,63,64], and thus this technique is considered the gold-standard. However, X-ray crystallography is a very laborious procedure that only allows for the identification of a monoclonal response, and consequently has a very low output. Another sophisticated technique is based on NMR that allows for a dynamic picture of the allergen:IgE antibody complex [63,65]. IgE epitope mapping by site-directed mutagenesis is based on systematic introduction of residue substitutions along the allergen, and a subsequent determination of the effect of each mutation on allergen recognition by IgE [56]. However, like X-ray crystallography, NMR and site-directed mutagenesis techniques only allow for identification of a monoclonal response [63,66]. Another approach is phage display technology which is based on the screening of a random peptide library, for affinity selection of peptides mimicking structures of epitopes bound by specific IgE antibodies, followed by competitive immune-screening with the specific allergen for elution of peptides of interest [67,68]. In order to predict the location of IgE binding epitopes on allergens in a structural context different *in silico* based methods are available [69–71]. In contrast to other approaches allowing for identification of conformational epitopes, this technique allows for the identification of IgE binding epitopes of a polyclonal antibody response as well as for patient-specific identification of amino acids contributing to the IgE binding [67,68]. Recent advances in coupling the phage display technique with high-throughput sequencing, has allowed a tremendous increase in the data output [68]. However, a massive challenge with the phage display technology is the notorious selection of unspecific allergen unrelated peptides, which necessitate the use of control subjects [68].

Clinical applicability of IgE and IgE binding epitope based approaches

As sIgE is the main player in food allergic diseases, great effort has been made in order to find biomarkers that discriminate between asymptomatic sensitization and a clinical relevant

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Table 2. Pros and cons of methods for IgE binding epitope identification.

Epitope mapping technique	Pros	Cons	References
Peptide-array	Allows for identification of a polyclonal response Easy method Large data output	Identification of only linear epitopes	[60–62]
X-ray crystallography	Allows for identification of both linear and conformational epitopes All types of interactions are realized Most accurate structural information	Difficult to obtain diffracting crystals Laborious method Low data output Identification of only monoclonal responses	[56,63,64]
NMR	Allows for identification of both linear and conformational epitopes A rather fast technique Dynamic behavior of the allergen–antibody complex can be investigated	Generally identification of only monoclonal responses Limited to allergens:antibody complexes of small sizes Low data output	[63,65]
Site-directed mutagenesis	Allows for identification of both linear and conformational epitopes	Identification of only monoclonal responses Laborious method Low data output	[63,66]
Phage display technology	Allows for identification of both linear and conformational epitopes Allows for identification of a polyclonal response Large data output	Laborious method Selection of unspecific allergen unrelated peptides	[67,68]

food allergy and between allergic phenotypes. Such biomarkers could be useful in predicting the course of the disease or the efficacy of therapeutic interventions.

Food allergy is a very heterogeneous disease according to clinical manifestations (severity and persistency). Consequently it would tremendously increase the diagnostic and therapeutic value of the available IgE and IgE binding epitope based approaches if the IgE binding characteristics, both at the allergen as well as the epitope level, could be correlated with the clinical phenotype. CRD facilitated the detection of patient-specific patterns at an allergen level. It revealed a broad heterogeneity in the allergen-specific responses between patients [72,73]. In some conditions this is helpful to understand the food allergic phenotype. In particular in peanut allergy both, the diversity as well as the recognition of specific allergens, such as Ara h 2, have been associated with severe peanut allergy [74,75]. In peanut allergy also cell-based approaches, such as BAT, have provided clinically meaningful data to predict food allergy [37]. Even though the applicability of cell-based assays in identifying a clinical relevant food allergy and the associated phenotype are only scarcely described, these could be promising future diagnostic and monitoring tools as the assays are based on the functionality of the raised IgE response rather than just the presence of sIgE.

Investigating the role of IgE binding epitopes in food allergy has involved the attempt to correlate patterns of IgE binding epitope recognition as well as the attempt to correlate individual epitope biomarkers with a clinically relevant food allergy and the associated allergic phenotype [7]. On the epitope level a great heterogeneity exists between individual patients, with each having their own unique pattern of IgE binding epitopes [62,67]. Further, IgE epitope mapping performed with the inclusion of alanine scan has revealed that patients reacting towards the same epitope may indeed react with heterogeneity at the amino acid level, revealing different patterns of amino acids contributing to the antibody binding [62]. However, the clinical relevance of the binding pattern at the amino acid level needs to be elucidated. Several studies suggest an association between IgE epitope diversity and persistency [31,76,77] as well as severity [31,78,79] of the food allergy. In milk and egg allergy the recognition of specific IgE binding epitopes has been suggested as biomarkers of persistency and/or severity [76,77]. On equal terms epitope mapping may be utilized in the monitoring of therapeutic efficacy [80]. Additionally, there has been an interest in the therapeutic utilization of epitope mapping [66,81], by means of modifying specific allergenic areas of the allergen or identifying new therapeutic targets [81]. Although all methods allowing for identification of IgE binding epitopes have limitations, epitope mapping could be a promising future tool for diagnosis and treatment of food allergic individuals.

Conclusion

New or improved approaches based on allergen sIgE, their binding sites or functionality have the potential to become accurate and trustworthy tools for diagnosis, prognosis and monitoring of therapeutic efficacy in food allergy and will add to our understanding of the etiology and pathology of this disease. However, more research is needed in order to

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invent tools providing accurate information on the course of the food allergic disease.

Conflict of interest

The authors have no conflict of interest to declare.

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