

The role of immunoglobulin E-binding epitopes in the characterization of food allergy

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Purpose of review

Allergen-specific immunoglobulin E (IgE) antibodies play a central role in food allergic reactions. IgE epitope mapping of food allergens may provide information regarding patient's clinical history and contribute to food allergy diagnosis/prognosis. The goal of this article is to review recent developments in the methods for IgE epitope mapping and the role of IgE-binding epitopes in the characterization of food allergy.

Recent findings

Recent studies have suggested a potential role for sequential IgE-binding epitopes as biomarkers for characterizing various phenotypes of food allergy. Studies of allergens in milk, peanut, egg and wheat have shown a correlation between IgE sequential epitope diversity and patients' allergy severity or persistence. Several informative epitopes in milk allergens have been identified as candidate biomarkers to predict the development of tolerance to milk. However, limitations with current methods of IgE-binding epitope identification need to be addressed before they can be applied in the diagnosis/prognosis of food allergy.

Summary

IgE epitope mapping has the potential to become an additional tool for the diagnosis/prognosis of food allergy and lead to a better understanding of the pathogenesis and tolerance induction of food allergy.

Keywords

epitope mapping, food allergy diagnosis/prognosis, immunoglobulin E-binding epitope

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Introduction

Food allergy is an emerging major public health problem that affects 3.5–4% of adults and 6–8% of young children in the USA [1]. The most common types of food allergies are mediated by allergen-specific immunoglobulin E (IgE) antibodies [2], which play a central role in allergic reactions. Cross-linking of membrane-bound IgE antibodies by the specific allergens results in activation and degranulation of effector cells (mast cells or basophils) with release of histamine and other inflammatory mediators, which together lead to the symptoms of allergic diseases. The groups of amino acids within allergenic proteins that are bound by IgE antibodies are called IgE-binding epitopes or B-cell epitopes.

Because allergen-specific IgE antibodies are the main effector molecules in the immune response to allergens, studies have focused on the identification of IgE-binding epitopes. Many IgE-binding epitopes from a variety of food allergens have been identified, which will undoubtedly contribute to the design of well tolerated immunotherapeutic agents [3,4]. Recent developments in the methods of epitope mapping have facilitated studies of

IgE-binding epitopes and their role in the characterization of food allergy. A positive relationship has been found between IgE epitope recognition and allergic patients' clinical sensitivity [5,6^{••}]. Efforts are now underway to search for informative IgE-binding epitopes as biomarkers of clinical severity, persistence or both of patients' allergic diseases [7,8]. IgE epitope mapping may provide an additional tool for allergy diagnosis and prognosis. In this review, we discuss the types of IgE-binding epitopes, the methods for epitope mapping, the role played by IgE-binding epitopes in the characterization of food allergy and the limitation or problem with current methods of IgE epitope mapping.

Types of immunoglobulin E-binding epitopes and methods for determining immunoglobulin E-binding epitopes

There are two types of IgE-binding epitopes, sequential and conformational in allergens [9]. A sequential (or linear) epitope is a sequence of contiguous amino acids that is recognized by IgE antibodies, whereas a conformational epitope comprises amino acids that line up because of the tertiary structure of an allergen and is recognized by IgE

antibodies. Sequential epitopes have been suggested to be more important in food allergens because food proteins are usually cooked, leading to heat denaturation and alteration in tertiary structure, and digested in the gastrointestinal tract, leading to further alteration and break-up of the tertiary structure before reacting with the immune system. In contrast, conformational epitopes are more relevant in respiratory allergens, although patients with oral allergy syndrome (OAS) [10], which is a pollen-associated food allergy in which patients experience symptoms in the mouth and throat following ingestion of raw fruits and vegetables, react to conformational epitopes because of cross-reactivity between food allergens and homologous pollen-related allergens.

Sequential epitopes

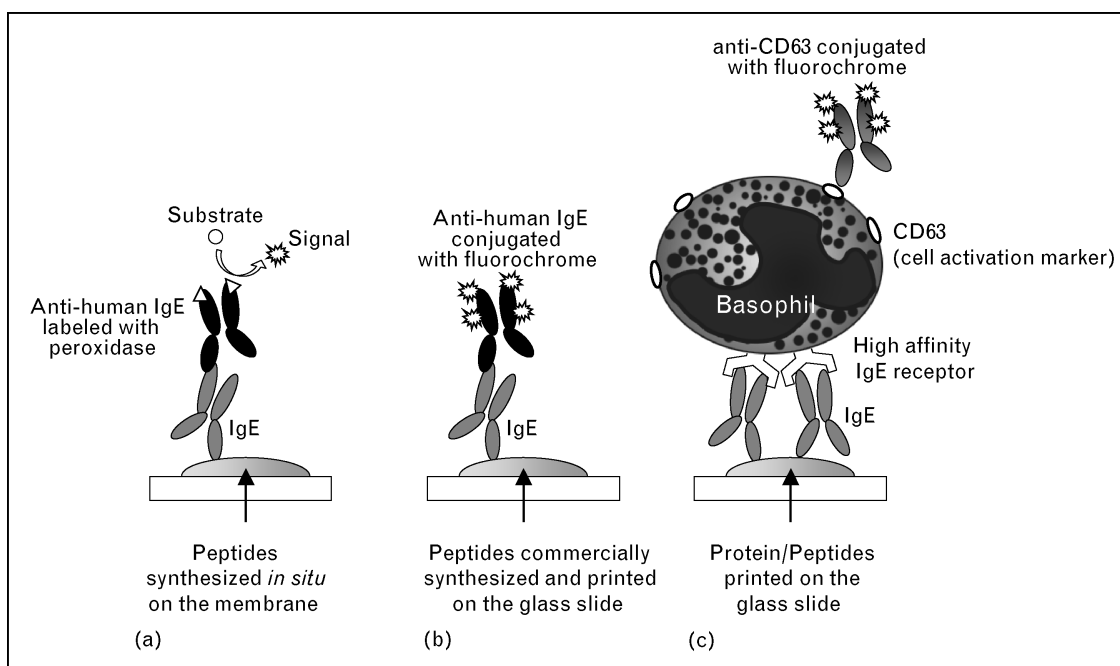
Compared with conformational epitopes, sequential epitopes are relatively easier to study, and many of them from different allergic sources are now characterized [11]. Recent findings that patients with persistent food allergy or a history of severe allergic symptoms recognized a higher ratio of sequential epitopes than conformational epitopes have highlighted the importance of IgE sequential epitopes [6^{••},12–16].

Methods of determining IgE sequential epitopes have developed over the last few years. The conventional method to localize B cell epitopes involved partial protease digestion or chemical cleavage at sites of specific

amino acids of the food allergen, followed by western blotting using patients' sera and amino acid sequencing of IgE-binding fragments [17,18]. For example, one allergenic epitope of codfish allergen, Gad c 1, was roughly located onto a duodecapeptide (AA no. 33–44) derived from trypsin digestion. The application of this fragmentation approach is restricted by the position of the cleavage sites of the allergens.

SPOT membrane-based immunoassay technology [13,19] was then introduced for allergenic epitope mapping (Fig. 1a). In this system, overlapping peptides covering the primary amino acid sequence of food allergens are synthesized *in situ* on a nitrocellulose membrane and then incubated with the patient's sera. The peptides bound by IgE antibodies are detected using antihuman IgE antibody labeled with peroxidase, followed by the addition of a chemoiluminescent substrate and exposure to radiographic film. This system is much more flexible than the fragmentation approach and can be used for any specific peptide sequence. Many of the available IgE sequential epitopes were mapped with this method [11], and currently, it is still being used for small-scale screening of peptides for epitope mapping. However, its peptide synthesis procedure involves many cycles of coupling, blocking and deprotection reactions on the nitrocellulose membrane. The percentage of peptide byproducts due to the inefficient coupling reactions becomes quite significant after several cycles and greatly affects the specificity

Figure 1 Comparison between SPOT membrane immunoassay (a), peptide microarray immunoassay (b) and basophil-mediated microarray immunoassay (c)



IgE, immunoglobulin E.

and background, that is, signal-to-noise ratio [20,21]. Other disadvantages of the SPOT membrane immunoassay include the large volume of patient sera that is required and the limited number of target peptides.

A novel immunoassay that utilizes the microarray platform is being developed to determine allergenic IgE-binding epitopes (Fig. 1b). The first application of this system was by Shreffler *et al.* [5] to determine IgE-binding epitopes of the major peanut allergens, Ara h 1, Ara h 2 and Ara h 3. The results of this methodology were found to be very consistent with those identified previously by SPOT membrane technology [22]. The high throughput platform of this system allows screening of thousands of target peptides in parallel using microliter quantities of serum, greatly reducing the biological sample cost of individual assays. Unlike the SPOT membrane technology, the overlapping peptides bound to the slide are commercially synthesized so that their purity can be strictly controlled with high-performance liquid chromatography purification and mass spectrometric analysis. The peptide spots bound by human IgE antibodies are detected using fluorescent-labeled antibodies followed by scanning with a microarray scanner, which is much more convenient than radiographic film development. In addition, peptide microarray provides quantitative data rather than semi-quantitative or qualitative results such as the SPOT technology, and its redundancy allows more robust replication and statistical approaches. Consequently, peptide microarrays may be more accurate and sensitive than SPOT membrane immunoassays. Epitope mapping of allergens from different sources are currently ongoing.

Initial studies have shown that the majority of IgE-binding epitopes identified are eight amino acids or longer [11], although peptides as short as five amino acids in wheat glutenin have been reported to be IgE epitopes [23]. Currently, the number of amino acids for an epitope is determined on the basis of the overlapping amino acids from peptides with positive binding to patients' IgE antibodies. However, the statistical definition of positive binding or reaction varies between studies, and the length of the epitopes accepted is therefore arbitrary. For example, when using the SPOT membrane technology, in the study of B cell epitopes in gliadin, Osman *et al.* [24] defined a peptide spot as positive if its optical density (OD), as measured on the developed radiographic film, was higher than the OD of the background. In other studies such as the study by Chatchatee *et al.* [25] of milk allergenic epitopes, a different arbitrary cut-off (i.e. OD of 0.2) to distinguish specific binding from nonspecific binding of sera from donors without milk allergy was applied.

Moreover, studies of milk [26] and peanut allergens [27] showed heterogeneous patterns of IgE antibody recog-

nition from different patients. In a study, Cocco *et al.* [26] showed that the amino acids critical for IgE binding varied among different patients. Likewise, it could be expected that the number of amino acids involved in epitopes may also vary between patients.

Conformational epitopes

Because of the limitation of available three-dimensional structures of food allergens and the difficulty of maintaining protein stability, studies of conformational epitopes are far behind those of sequential epitopes. Conformational epitopes are important for allergens involved in OAS and respiratory allergy. Moreover, for some other allergens, such as allergens from the lipid transfer protein family, they possess compact folding and high stability that enables them to survive gastric digestion. Chances are that some of these proteins may interact with the immune system, whereas part of their tertiary structure remains intact, and thus, conformational epitopes might be involved in some systemic food allergic reactions [28,29].

One approach to study conformational IgE-binding domains was to use molecular biological methods for production of recombinant allergens [30]. For example, Alcocer *et al.* [31] constructed several chimeric proteins by structural replacement of domains from the allergenic Brazil nut 2S albumin into the structurally similar but much less allergenic homolog sunflower seed 2S albumin. Circular dichroism spectra of the chimeric proteins showed a similar α -helical secondary structure as their native counterparts, indicating that the secondary structures have not been affected during protein expression. By screening a panel of Brazil nut allergic individuals, a structural IgE-binding epitope of Brazil nut 2S albumin was mapped to a helix-loop-helix region.

The recently developed mimotope mapping technique is another option for mapping conformational epitopes [32]. Using random peptide phage-display libraries, peptides are generated and screened with purified allergen-specific IgE. The selected peptides that mimic the binding sites of the allergen but do not correspond to its sequential sequence are known as mimotopes. The resulting mimotope can be mapped onto the three-dimensional structure of the allergen using computational biological techniques. Using this technique, IgE conformational epitopes have been identified from parvalbumin [33] and peach Pru p 3 [34*].

The role of immunoglobulin E epitopes in the characterization of food allergy

With the development of methods for determining IgE-binding epitopes, recent studies have suggested a potential role for sequential IgE-binding epitopes as

biomarkers for characterizing various phenotypes of food allergy.

Relationship between immunoglobulin E-binding epitope recognition patterns and clinical allergy

In-vitro measurement of allergen-specific serum IgE [35], along with the patient's clinical history, is used to predict the clinical reactivity of food allergic patients. Unfortunately, many patients do not have convincing histories, food-specific IgE antibody levels are low or both, leaving considerable doubt about the patient's allergic status. Consequently, allergen-specific IgE levels cannot replace the double-blind, placebo-controlled oral food challenge (DBPCFC) [36], which remains the 'gold standard' for food allergy diagnosis, although it carries some risk and is time-consuming [37]. In addition, the majority of pediatric patients with some forms of food allergy (e.g. milk, egg, wheat, soy) will outgrow their food allergy during childhood. The natural history of tolerance development and how it varies between patients with different allergic histories is poorly understood.

Two important steps in the progressive differentiation of antigen-specific B cells are clonal expansion and affinity maturation in which selected clones with higher affinity receptors expand and further diversify [38,39]. In allergy, that may lead to IgE antibody clones with higher diversity and affinity. Thus, characterization (i.e. concentration, diversity and affinity) of antibody clones in the IgE repertoire may reveal a patient's allergic history, current status or both. IgE measurement for specific food allergens has limited application as it only measures the overall concentration of antibody clones in patient's IgE repertoire toward the allergen but not other features. Therefore, measurement of specific IgE to allergenic epitopes, which represents allergen-specific IgE antibody diversity, may provide additional information regarding reaction severity and persistence of allergy. With the currently available IgE epitope mapping methods (i.e. SPOT membrane technology and peptide microarray), several studies have been performed to investigate the relationship between epitope-specific IgE and clinical allergic symptoms, as well as the prediction of persistent food allergy.

In a retrospective study [5] of peanut allergic patients, higher IgE sequential epitope diversity to peanut allergens, Ara h 1, Ara h 2 and Ara h 3, was found in patients with a history of more severe allergic reactions to peanut. In addition, sensitization of effector cells with sera containing more diverse IgE antibodies conferred greater effector response to specific allergens in a basophil activation assay. The correlation between IgE sequential epitope diversity to the same allergens and clinical

sensitivity was further investigated by Flinterman *et al.* [6^{••}], but this time, the clinical sensitivity of 24 peanut-sensitized children was determined prospectively by means of double-blind, placebo-controlled peanut challenges. A positive correlation ($r=0.6$) between IgE epitope diversity, as represented by the number of epitopes recognized, and clinical sensitivity was confirmed in this study. The authors also analyzed the serum samples from most of the patients obtained 20 months after challenge and showed that their epitope-recognition patterns were essentially stable.

A correlation between IgE sequential epitope diversity and allergy severity or persistence was found in patients with allergy to milk [13,25,40] and egg [14,41]. Several studies to determine IgE-binding epitopes of different milk allergens, including alpha(s1)-casein, beta-casein and kappa-casein and alpha-lactoglobulin and beta-lactoglobulin, have compared the epitope recognition patterns between patients less than 3 years of age, who were likely to outgrow their milk allergy and had low levels of specific IgE, and older patients with persistent milk allergy and high levels of specific IgE antibodies to cow's milk. All of these studies showed that the majority of patients in the older age group recognized a much greater number of IgE-binding epitopes than the younger patients, indicating an association between recognition of certain epitopes and clinical symptoms of cow's milk allergy. Similarly, patients with persistent egg allergy developed more IgE antibodies against sequential and conformational epitopes of ovomucoid and ovalbumin, with four IgE sequential epitopes of ovomucoid recognized by all the patients with persistent egg allergy but not by any of the patients with transient allergy.

In wheat allergy, patients with more severe reactions to wheat (i.e. wheat-dependent exercise-induced anaphylaxis) also showed significant IgE reactivity to gliadin sequential epitopes, whereas patients with atopic dermatitis or eczema showed very low or no reactivity [16].

Identification of informative epitope as biomarker in allergy diagnosis/prognosis

Studies evaluating milk allergenic (IgE-binding) epitopes not only associated high epitope diversity with long-lasting allergy but also demonstrated that sequential epitopes recognized by IgE antibodies in older patients with persistent allergy differ from those in younger children who are likely to outgrow their allergy. It was shown that certain epitope-specific IgE antibodies were present at a very early age in patients who developed persistent food allergy. Therefore, these epitopes, labeled as 'informative' epitopes, may be useful as a biomarker of persistent food allergy and predict the clinical outcome of the allergy.

Several milk IgE sequential epitopes have been identified as candidate biomarkers of severity, persistence or both of patients' allergic conditions. Using sera from 10 patients with persistent cow's milk allergy and 10 patients with transient milk allergy, Jarvinen *et al.* [12] identified six IgE-binding epitopes that were recognized by most of the patients with persistent allergy but not bound by IgE from any of the patients who 'outgrew' their allergy, that is, transient allergy. Among these epitopes, three appeared to be more 'informative', as the presence of IgE antibodies against at least one of these epitopes identified all patients with persistent milk allergy. The utility of informative epitopes in predicting persistent milk allergy was assessed by a prospective study including patients with challenge-proven milk allergy but with different natural histories of milk allergy: patients with persistent milk allergy ($n = 45$; median age, 8 years) and patients who outgrew their milk allergy at an earlier ($n = 15$; median age, 3 years) or later age ($n = 14$; median age, 8 years). Patients with persistent milk allergy showed significantly higher IgE level to peptides containing the informative epitopes than those who outgrew milk allergy, confirming that IgE antibodies to these informative epitopes can be used as a marker of persistent milk allergy [8].

Cerecedo *et al.* [7^{*}] also identified several IgE sequential epitopes that showed differential binding recognition patterns between patients who were reactive to milk and those who were tolerant, as verified by oral milk challenges. Only one of the epitopes identified overlapped with those previously identified by Jarvinen *et al.* [12]. This is possibly because of differences in the patient populations studied, epitope mapping methods, methods of data analysis or all. In the study, Cerecedo *et al.* [7^{*}] used the peptide microarray immunoassay in patients with milk-specific IgE levels mostly less than 2.5 kU_A/l, whereas most patients with persistent milk allergy in the study by Jarvinen *et al.* [12] had milk-specific IgE levels of more than 100 kU_A/l, and IgE antibodies to each epitope were identified using SPOT membrane technology for epitope determination.

Limitation or problem of using immunoglobulin E-binding epitopes in allergy diagnosis/prognosis

Although IgE epitope mapping provides an additional tool with great flexibility for the study of food allergy, its application in the determination of diagnosis/prognosis of food allergy is limited.

High-power quantitative studies in which larger number of patients are required

Compared with allergen-specific IgE measurements, determining epitope-specific IgE provides more infor-

mation regarding the patient's clinical history and thus has a broader application in diagnosis/prognosis of food allergy. However, the epitope binding patterns are heterogeneous between patients, and there is still an overlap between epitope-specific IgE values from different patient groups tested [8,27]. One example is the overlap in IgE epitope recognition between reactive and tolerant patients in the study by Cerecedo *et al.* [7^{*}]. Although 10 epitopes have been identified as recognized by more than 75% of reactive patients ($n = 16$) and significantly associated with the reactive group, they were also recognized by a small percentage (13.3–40%) of tolerant patients ($n = 15$). In the study by Beyer *et al.* [8] to assess the utility of candidate informative epitopes, there were also overlaps in the epitope-specific IgE levels between patient groups with a different natural history of milk allergy, although they were significantly higher in patients with persistent milk allergy. Studies performed so far suggest that it is unlikely to assign patients to different groups solely on the basis of only one or two epitope biomarkers. Possibly a combination of several epitope biomarkers would be more informative. Bioinformatic methods such as supervised machine learning and classification may be useful in developing prediction models using a combination of these biomarkers [42]. However, high-power quantitative studies with hundreds of patient samples are required to build a robust and generalizable model.

Affinity or avidity of epitope-specific immunoglobulin E binding

As mentioned earlier in this review, affinity (the binding strength between IgE antibodies and allergen or allergenic epitopes) may be one of the factors involved in pathogenesis of allergic diseases. Most studies to date have measured only the concentration of IgE antibodies to either allergens or allergenic epitopes, but the affinity of IgE binding is rarely considered.

One method to measure the affinity, or avidity (the overall binding strength of multivalent antibodies to an antigen with many antigenic determinants), is to use thiocyanate, a chaotropic agent that interferes with noncovalent binding, to remove low-affinity IgE bound to an allergen. Using this method, El-Khouly *et al.* [43] have shown that the avidity of peanut-specific IgE to Ara h 2 was weakly correlated with the severity of peanut allergy. Apart from thiocyanate, pH reduction or the use of competing proteins or peptides to elute IgE antibodies of lower affinity bound to antigen may also be useful [32]. This method could be combined with the epitope mapping tools to measure the affinity or avidity of epitope-specific IgE.

A systematic study of IgE antibody concentration, clonality and affinity or avidity was performed by Christensen

et al. [44**]. In this study, a panel of 31 recombinant IgE antibodies specific for the major house dust mite allergen, Der p 2 (rDer p 2), was developed and characterized using a chip-based surface plasmon resonance technology, which measures antigen and antibody interaction in real time and provides information regarding concentration, affinity and clonality for each antibody. A basophil activation test was performed by sensitizing human basophils with different combinations of rDer p 2 antibodies, mimicking allergic IgE repertoires of known compositions. The authors demonstrated that IgE antibody affinity, total IgE concentration, ratio of allergen-specific IgE to total IgE and IgE clonality are all correlated with effector cell degranulation.

Clinical relevance of the in-vitro immunoglobulin E epitope mapping

Application of IgE epitope mapping is also limited by the clinical relevance of in-vitro IgE binding. Positive IgE binding merely indicates sensitization to a peptide or protein, but this binding does not necessarily imply that the allergens are cross-linking IgE on mast cells or basophils *in vivo* [10]. IgE binding to single epitopes of allergenic components might therefore not be sufficient to provoke clinical symptoms. Therefore, several studies of IgE-binding epitopes have tried to assess in-vitro IgE binding with a more biological system, such as the basophil activation test in the studies described above by Christensen *et al.* [44**] and the peanut allergenic epitope study by Shreffler *et al.* [5]. A basophil-mediated microarray immunoassay (Fig. 1c) was recently developed, in which the printed spots of protein or peptide-containing allergenic epitopes were not detected with patient's serum IgE but with human basophils passively sensitized with patient's serum [45]. This system combines the diversity of microarray technology with the assessment of biologic activity in a cell-based assay and might enable a better estimation of the clinical relevance of in-vitro IgE binding.

Conclusion

Development of new technologies (i.e. peptide microarray, phage-display library) for epitope mapping has greatly facilitated studies of IgE-binding epitopes and their role in the characterization of food allergy. Although there are still several limitations with current studies of IgE-binding epitopes, a correlation has been found between patients' IgE epitope recognition and allergy severity or persistence, with several informative epitopes identified as candidate biomarkers to predict the development of tolerance. In the future, IgE epitope mapping has the potential to become an additional tool for the diagnosis/prognosis of food allergy and lead to a better understanding of the pathogenesis and tolerance induction of food allergy.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 391).

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