Beyond Skin Testing: State of the Art and New Horizons in Food Allergy Diagnostic Testing

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KEYWORDS

• Allergy • Food • IgE-mediated • Diagnosis • Microarray
• Molecular • Component-resolved diagnosis • Basophils

Food allergy represents a major health problem in infants and children, with an increasing prevalence. Recent epidemiologic studies based on objective diagnostic methods estimate that 1% to 10.8% of the general population suffer from food allergy.[1] The term "food allergy" refers to adverse immunologic reactions to food and should be distinguished from food intolerances that do not have an immune basis, such as a lactase deficiency. However, up to 35% of the population in Western countries self-report food allergy, indicating the magnitude of the problem and the need for appropriate diagnostic methods.[1,2] Accurate diagnosis of food allergy is important not only to prevent serious or even life-threatening reactions but also to avoid unnecessary dietary restrictions that could place individuals at risk for nutritional deficiencies and growth deficits.

In the diagnosis of food allergy no single investigation is fully reliable, and a stepwise approach is recommended by the international guidelines.[3] After a detailed history and physical examination, the allergy workup may be completed by in vivo and/or in vitro allergy tests, that is, skin-prick tests and/or measurement of food-specific immunoglobulin E (IgE) antibodies. Diagnostic cutoff values have been proposed to predict the likelihood of reactivity to various specific foods (**Table 1**). However, none of these diagnostic parameters have achieved sufficiently high predictive values, and thus most patients still need to undergo clinician-supervised oral food challenges

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Table 1
Laboratory tests to assess the likelihood of obtaining a positive or negative oral food challenge in young children

	Serum Food-IgE (kU_A/L)[a]	
Food	**≈95% Positive**	**≈50% Negative[b]**
Cow's milk	≥15[89] ≥5 if younger than 1 y[91]	≤2[90]
Egg white	≥7[89] ≥2 if younger than 2 y[92]	≤2[90]
Peanut	≥14[89]	≤2 with and ≤5 without history of peanut reaction[93]
Fish	≥20[89]	

[a] Phadia ImmunoCAP.
[b] In the authors' experience, children with about 50% chance of experiencing a negative challenge are the optimal candidates for an office-based oral food challenge. However, serum levels of food-specific IgE antibodies are not absolute indications or contraindications to performing an oral food challenge. Laboratory test results have to be always interpreted in the context of clinical history.
Modified from Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, et al. Work Group report: oral food challenge testing. J Allergy Clin Immunol 2009;123(Suppl 6):S365–83; with permission.

(OFC). However, OFC are resource consuming and are associated with a risk for severe anaphylaxis. New testing methodologies are required to assess the presence and severity of a food allergy, as well as the resolution of the disease. At present, research efforts focus on improving diagnostic tests and on developing new tools that provide better prognostic performance. This review discusses several promising novel approaches for the diagnosis of IgE-mediated food allergy and their potential clinical applications.

MOLECULAR DIAGNOSIS IN FOOD ALLERGY

Current tests used to diagnose IgE-mediated food allergy perform relatively poorly in differentiating asymptomatic sensitization from true allergic reactions because they are typically performed with crude allergen extracts. Indeed, these extracts are difficult to standardize and consist of a mixture of allergenic and nonallergenic components, some of them cross-reacting with homologous proteins from other sources (ie, cross-reactive carbohydrate determinants) (**Table 2**).[4,5] Molecular diagnostic technologies have been recently introduced into allergy research as promising tools. Instead of measuring the IgE response to complex allergen extracts, specific responses at the level of individual allergenic proteins (component-resolved diagnosis [CRD]) or the IgE-binding epitopes of those allergens (epitope mapping or profiling) are evaluated.

Component-Resolved Diagnosis

The term "component-resolved diagnosis" has been used to designate diagnostic tests based on pure allergen proteins, which are either produced by recombinant expression of allergen-encoding complementary DNA or by purification from natural allergen sources.[6] For the most common foods, many allergenic proteins have been identified, sequenced, and cloned. Recent advances in proteomics research, including 2-dimensional gel electrophoresis, mass spectrometry, protein arrays, and improved bioinformatics, have largely expanded the library of known food components, although identification of new allergens is increasing steadily.[7,8] The benefits and problems of the different allergen preparations available are outlined in **Table 2**.

Table 2
Benefits and problems of allergen preparations used for in vitro diagnostics

	Natural Extracts	Native Allergens	Recombinant Proteins
Advantages	Easy to prepare Ideally, all allergenic proteins are present	Enabling of CRD Native protein structures are mostly preserved Presence of all natural isoforms and posttranslational modifications	Enabling of CRD and application of a single isoform Lack of impurities with other food proteins Standardization of amount and structural characteristics
Disadvantages	Standardization problems caused by the natural variability of active ingredients and endogenous degradation that also can cause low assay sensitivity Complex mixtures of allergenic and nonallergenic components sometimes resulting in low assay specificity	Laborious preparation Yield depends on composition of source material Risk of variable batch composition caused by different copurification yields of isoforms Risk of low-level contamination with other allergens from the same source and purification artifacts	Laborious preparation Proteins can be unfolded or partially unfolded and might not be properly modified after translation Risk of low-level contamination with components of the expression system and purification artifacts

Abbreviation: CRD, component-resolved diagnosis.

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Potential Clinical Application of CRD

Studies comparing diagnostic performances of CRD to traditional allergy tests, that is, skin-prick tests and specific IgE, suggest that component testing could improve specificity for several foods. For example, a recent study evaluated the effectiveness of CRD to distinguish between patients allergic to peanuts and those sensitized but clinically tolerant.[9] By using specific IgE to the component protein, Ara h 2, with a cutoff point of 0.35 kU_A/L, 97.5% of the population was correctly classified, and all patients allergic to peanut were correctly identified. The misclassification rate using a whole peanut-specific IgE level of 15 kU_A/L was about 18% in this study.

Similarly, the value of specific IgE antibodies to omega-5-gliadin (Tri a 19) has been evaluated in the diagnosis of wheat allergy. Although Tri a 19 was previously identified as a major allergen in wheat-dependent exercise-induced anaphylaxis,[10] recently it has been shown to be a significant allergen in young children with immediate allergic reactions to wheat.[11,12] In a recent study, the level of specific IgE to Tri a 19 was related to the challenge outcome in wheat-sensitized children and to the severity of the reaction.[12] Moreover, specific IgE to Tri a 19 had superior performance to that of wheat-specific IgE for the prediction of clinical reactivity to wheat. However, not all investigators have found it specific.[13]

Measurement of specific IgE to individual components may also provide important additional information to identify different clinical phenotypes of food allergy. In children allergic to egg, greater levels of ovomucoid-specific IgE were found in those reacting to baked egg than in those tolerant to baked egg and regular egg.[14] Low levels of IgE against ovomucoid indicated a low risk of reaction to baked egg. Likewise, the authors found that casein-specific IgE has superior accuracy for predicting baked milk reactivity compared with cow's milk–specific IgE. (Caubet JC, Nowak-Węgrzyn A, Moshier E, et al. Utility of casein-specific IgE levels in predicting reactivity to baked milk. Submitted for publication.)

Furthermore, CRD may be useful in predicting the severity and/or persistence of the disease. High levels of casein-specific IgE antibodies have been identified as a risk factor for persistence of cow's milk allergy[15,16] and for more severe allergic reactions, especially in asthmatic children.[17] Similarly, it has been shown in 2 different studies that children with persistent egg allergy had significantly higher ovomucoid-specific IgE levels than those who outgrew their egg allergy.[18,19] A favorable prognosis was associated with the absence or a decline in ovomucoid-specific IgE titers.[18]

Determining allergen sensitization profiles could help to assess the risk of cross-reactive allergies to other food sources and to avoid unnecessary exclusion diets. The most illustrative example is patients with fish allergy. Because of a high degree of cross-reactivity between parvalbumin from different fish species,[20] patients sensitized to a fish parvalbumin (eg, Gad c 1 from cod[21] and Cyp c 1 from carp[22]) are likely to react to a range of different fish species. However, some patients allergic to fish can tolerate some fish species while being allergic to others.[23] A recent study suggests that the different expression level of parvalbumin in specific species might explain tolerance to some species such as swordfish.[24] The differences in clinical response to fish species might also be explained by reactivity to allergens other than parvalbumins.[25] A better understanding of the allergenic characteristics of different fish species helps to better predict cross-reactivity[26,27] and improve the management of patients allergic to fish.

In addition, component testing may help to differentiate between sensitization caused by cross-reactivity with pollens and systemic clinical allergy (**Table 3**). In peanut allergy, for example, the presence of specific IgE antibodies to Ara h 8 (a Bet v 1 homolog) is a marker for birch-pollen–related reactions to peanut. For example,

Table 3
Plant food allergens classified according to their cross-reactive potential

Food	Pollen Cross-Reactive Components[a]	Lipid Transfer Protein	Pollen Non–Cross-Reactive Components[b]
Peanut	Ara h 8[c] Ara h 5[d]	Ara h 9	Ara h 1; Ara h 2; Ara h 3 Ara h 4; Ara h 6; Ara h 7
Hazelnut	Cor a 1[c] Cor a 2[d]	Cor a 8	Cor a 9 Cor a 11
Soybean	Gly m 4[c] Gly m 3[d]	Gly m 1	Gly m 5 Gly m 6
Wheat	Tri a 12[d]	Tri a 14	Tri a 19 (ω-5 gliadin) Tri a 21 (α gliadin) Tri a 26 (high–molecular weight glutenin) Tri a 28 (α-amylase inhibitor dimer 0.19)

[a] Birch-tree pollen, Timothy grass pollen for wheat.
[b] Storage seed proteins, albumins, and globulins.
[c] PR10 proteins.
[d] Profilin.

among children selected from a large birth cohort, peanut allergy symptoms were reported in 87% of the children with IgE reactivity to pollen-unrelated Ara h 1, 2, or 3, but not to Ara h 8 (n = 46), compared with 17% of children with IgE reactivity to Ara h 8, but not to Ara h 1, 2, or 3 (n = 23).[28] Moreover, patients sensitized to Ara h 1, 2, or 3 have been shown to have more severe symptoms.[29]

Like peanut allergy, IgE-mediated allergy to soy may be the result of primary sensitization to soy but could also result from cross-reactivity to birch-related tree pollen and a variety of legumes.[30–34] The presence of Gly m 5–specific and Gly m 6–specific IgE is a marker of primary sensitization associated with a higher risk of severe reactions.[31,32] Sensitization to Gly m 4 is common in patients allergic to birch pollen and is often associated with local reactions, although systemic reactions may also occur.[33,34]

Allergen Components on Microarray

In the United States, the allergen components are commercially available using the ImmunoCAP system (Phadia AB, Uppsala, Sweden). In Europe, protein microarray has recently been introduced for measuring specific IgE and is commercialized in the form of the ImmunoCAP-ISAC, Immuno Solid-phase Allergen Chip (VBC Genomics, Vienna, Austria; Phadia, Uppsala, Sweden).[35,36] It currently has 112 native/recombinant component allergens from 51 allergenic sources. This technology has 2 main advantages: it simultaneously assesses specific IgE to different components and requires very small amounts of sera, which is especially relevant in children. Moreover, ImmunoCAP-ISAC can be considered a cost-efficient approach because it delivers results for more than 100 components.

Ott and colleagues[37] evaluated the clinical performance characteristics of this assay regarding the outcome of the OFC for suspected allergy to cow's milk (n = 85) and eggs (n = 60), and found no advantage over the usual diagnostic tests, that is, skin-prick test and whole protein–based specific IgE. Although the diagnostic capability was not enhanced with the use of CRD, the investigators recommended the use of microarrayed allergen components as a minimally invasive tool because of the low quantity of serum required for analysis.

Using a customized version of the ISAC microarray, D'Urbano and colleagues[38] also investigated children with suspected cow's milk allergy and egg allergy, comparing allergen components with OFC. The results indicated that serial testing of specific IgE and microarray components had a clinical performance very close to that of the OFC. These investigators proposed to use the microarray as a second-level assay if the level of specific IgE is above 95% of the positive predictive value.[39] This approach could lead to a decrease in the number of the OFC to be performed.

Recent studies have also provided interesting results on microarray testing for the diagnosis of peanut,[9] wheat,[40] and milk allergy,[41] as well as for the diagnosis of oral allergy syndrome to apple.[42]

Using the same platform, more significant information could be obtained. For example, it is theoretically feasible that by spotting different concentrations of allergens on the chip, relative IgE antibody affinity can be determined.[43] Moreover, the parallel determination of different antibody isotypes (IgA, IgM, IgG, and IgE) using microarrays seems to offer promising results,[44] even when attachment to the microarray is achieved using whole food extracts.[45] A drawback of CRD microarrays is the risk of overdiagnosis and misinterpretation of the complex results of such tests.[46] Well-designed large-scale studies from different geographic areas are needed to evaluate the practical use of allergenic components in food-allergic patients.

Role of Epitope Mapping in the Diagnosis of Food Allergy

Food allergens must at least partially survive digestion and absorption from the gastrointestinal tract to be immunogenic, which has led to the hypothesis that individuals who generate IgE antibodies recognizing a greater number or a specific pattern of sequential epitopes (eg, those not easily destroyed by denaturation and partial digestion) are more likely to have clinical allergy rather than asymptomatic IgE sensitization.[47] Furthermore, the importance of recognizing sequential IgE-binding epitopes in the persistence and severity of allergy has been highlighted in several studies on milk,[48–50] peanut,[51,52] egg,[19,53] and wheat allergens.[54] For example, Vila and colleagues[55] found higher levels of IgE antibodies to specific sequential epitopes from casein in children who have persistent cow's milk allergy in comparison with those who were to develop tolerance.

In the past, epitope mapping was mainly performed using SPOT membrane-based immunoassays[48,49,56] whereby peptides were synthesized on a nitrocellulose membrane and then incubated with the patients' sera. However, synthesis of large numbers of peptides is relatively error prone, time consuming, labor intensive, and expensive and has limitations because of the specific chemistry of the method. A large volume of serum is required, and there is also a limitation of the number of targeted peptides. With the development of microarray technology and evolution in peptide synthesis techniques, peptide microarray-based immunoassays for epitope mapping of allergens may be the next step. Recently, several clinical studies on milk,[57,58] peanut,[51,52] and shrimp allergy[59] provided promising results, demonstrating that greater IgE epitope diversity and/or higher affinity were associated with clinical phenotype and/or severity of allergy. In the future, this assay might be useful for predicting the outcome of food allergy and for identifying patients at risk for persistent allergy as potential candidates for proactive treatment. However, technical issues and limitations need to be addressed before clinical use is attempted.

FUNCTIONAL ASSAYS

Basophil Activation Testing

Basophils represent a significant effector population in allergic pathogenesis. Because they can be stimulated ex vivo, they provide the theoretical potential of measuring

a biological allergic response, more so than specific IgE.[60,61] The first approach to basophil functional responses was the histamine release test, but this has remained controversial due to its insufficient sensitivity and specificity.[62,63] Several groups proposed using flow cytometry to identify the population of basophils and measure their activation based on upregulation of cell-surface molecules (eg, CD63 and CD203c).[64–66] The basophil activation test (BAT) is increasingly under investigation.[67]

Recently, based on 36 prospectively recruited patients, Rubio and colleagues[68] showed that BAT was a better predictor of milk allergy using challenge outcomes as the gold standard. It was also observed that children with clinical sensitivity to milk-containing baked products had greater basophil reactivity than tolerant children.[66] Another recent study examined the performance of BAT for predicting challenge outcome in a group of 71 children with egg or milk allergy previously diagnosed by challenge outcomes or convincing history.[69] These investigators found that assessment of food antigen–induced CD203c expression on basophils is useful to determine whether children will outgrow food allergy as well as to make decisions regarding whether or not to perform OFC. Other studies suggest that BAT is comparable to skin-prick tests or specific IgE levels in its ability to distinguish clinical allergy from sensitization in patients with food-pollen allergy syndrome.[70–73]

Recently a few papers have been published in which the BAT is activated using purified or recombinant components.[74–77] For example, Erdmann and colleagues[72] investigated the diagnostic value of BAT with recombinant allergens (Mal d 1, Dau c 1, and Api g 1) for the diagnosis of apple, carrot, or celery allergy in patients allergic to birch. The investigators found high specificities that were comparable to those of specific IgE to apple, carrot, and celery, but the sensitivities were lower in comparison with prick-to-prick testing using fresh fruits or vegetables. In the future, in analogy to CRD, the BAT as functional test may be used to define a patient's sensitization profile, using purified or recombinant allergen components, facilitating the discrimination between true allergy and clinically irrelevant sensitization to cross-reactive molecules.

Evaluation of T-Cell Responses

T-cell responses to food allergens have also been evaluated in the diagnosis of food allergy. Food-allergic patients in general have higher proliferative responses than sensitized patients or healthy controls, suggesting an intrinsic excessive reactivity of the T cells in food-allergic patients.[78] However, lymphocyte proliferation assays are neither diagnostic nor predictive of clinical reactivity in individual patients with food allergy.[79,80]

More specific analysis of allergen-specific T-cell responses may be useful to distinguish between sensitization and clinically relevant allergy. Recently, Flinterman and colleagues[81] used the CRD approach to characterize peanut-specific T-cell responses in patients allergic to peanuts (n = 18), peanut-sensitized patients (n = 7), and nonallergic control patients (n = 11). The T-cell response to crude peanut extract was stronger in children with peanut allergy than in those with peanut sensitization or without peanut allergy. Only the children with peanut allergy had detectable interleukin-13 production in response to major peanut allergens (Ara h 1, Ara h 3, and Ara h 6). Although T-cell subset CRD is unlikely to displace OFC as the gold standard, if reproduced these results could open a new perspective on the diagnosis of food allergy.

OTHER ASSESSMENT

Serum-Specific IgG Antibodies

Based on studies from the 1980s indicating that antigen-specific IgG_4 could induce histamine release from basophils,[82] testing for blood IgG_4 has been increasingly

performed with screening for hundreds of food items in patients with suspected food allergy and intolerance. Testing for food-specific IgG typically yields multiple positive results, which often represents a normal immune response to food. Indeed, specific IgG_4 antibodies are not predictive of food allergy,[83] and national and international guidelines do not recommend testing of IgG_4 to food in the allergy workup.[3]

On the other hand, emerging data from immunotherapy trials suggest that the IgG_4 immunoglobulin class may play a protective role, serving as blocking antibodies, in tolerance development.[84,85] Because the balance between allergen-specific IgE and IgG_4 production may affect whether clinical allergy or tolerance develops, the determination of the ratio of specific IgE/IgG_4 may be more useful than the absolute amount of IgG_4 for assessing the ongoing status of food sensitization. For example, measurement of the specific ratios IgE/IgG_4 to ovalbumin and/or ovomucoid has been shown to be useful in following the development of tolerance and outgrowing egg allergy in research studies.[86,87] These data need to be confirmed in further studies.

Other Nonvalidated Tests

Several other methods have been evaluated for the diagnosis of food allergy, including facial thermography, gastric juice analysis, endoscopic allergen provocation, hair analysis, applied kinesiology, provocation neutralization, electrodermal test (Vega), and mediator release assay (lifestyle, eating, and performance diet). However, there is a lack of evidence demonstrating that any of the tests have diagnostic value in food allergy.

SUMMARY

Improved interpretation of allergic testing facilitates the diagnosis of food allergy and eliminates unnecessary OFCs. Research efforts are focused on improving diagnostic tests and on developing tests that have a better prognostic performance. Molecular diagnostic assays are especially promising and could significantly improve the management of food allergic patients by providing a more individualized medical approach to care. However, these methods still need to be validated against OFCs, considered the gold standard, in large-scale studies and in different geographic regions. Functional assays, such as BATs, particularly in combination with allergen components, might also be useful and need to be further investigated. In the future, coupling the diversity of a microarray approach with the potential functionality and biological activity of a cell-based test may result in a new system to improve the diagnosis of food allergy.[88]

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