Potential, pitfalls, and prospects of food allergy diagnostics with recombinant allergens or synthetic sequential epitopes

Stephan Steckelbroeck, PhD,[a] Barbara K. Ballmer-Weber, MD,[b] and Stefan Vieths, PhD[a] *Langen, Germany, and Zurich, Switzerland*

This article aims to critically review developments in food allergy diagnostics with regard to the verification of specific IgE antibodies and the identification of the responsible allergens. Results of IgE-binding tests with food extracts are hampered by cross-reactive proteins, low-quality test agents, or both. Specificity can be increased by defining adequate cutoff values, whereas sensitivity can be improved by using high-quality test agents. IgE-binding tests with purified allergens enabled reliable quantification of allergen-specific IgE titers, with higher levels found in individuals with food allergy compared with individuals without food allergy. However, the overlap in individual test reactivity between allergic and nonallergic subjects complicates interpretation. Recombinant allergens and synthetic sequential epitopes enabled detection of sensitization profiles, with IgE specific to several allergens and substructures now being suggested as markers of severity, persistence, or both. However, high-power quantitative studies with larger numbers of patients are required to confirm these markers. IgE-binding tests merely indicate sensitization, whereas the final proof of clinical relevance still relies on family/case history, physical examinations, and provocation tests. Novel technologies promise superior diagnostics. Microarray technology permits simultaneous measurement of multiple IgE reactivities regarding specificity, abundance, reactivity, or interaction. Improved functional tests might enable reliable estimation of the clinical relevance of IgE sensitizations at justifiable expenses. (J Allergy Clin Immunol 2008;121:1323-30.)

Key words: *Food hypersensitivity, component-resolved diagnosis, recombinant food allergen, sequential epitope, immunoblot inhibition, skin prick test, IgE quantification, sensitivity, specificity, cross-reactivity*

Abbreviations used
sIgE: Allergen-specific IgE antibody
SPT: Skin prick test

Epidemiologic studies based on food challenges indicate that 1% to 10.8% of the general population have immune-mediated nontoxic food hypersensitivity, which is the most common trigger of anaphylaxis in young age.[1,2] Food allergy includes IgE-mediated and non–IgE-mediated syndromes, where IgE-mediated manifestations are responsible for the majority of food-induced, immediate-type, immune-mediated hypersensitivity reactions.[3] The development of an IgE-mediated response to food requires a series of molecular and cellular interactions, which involve antigen-presenting cells, T lymphocytes, and B lymphocytes.[4,5]

Depending on the route of sensitization, food allergy is the result of either genuine reactivity to comestibles through the gastrointestinal tract (class I food allergens) or secondary sensitization to cross-reactive food allergens as a consequence of the initial reactivity to homologous pollen-related allergens (class II food allergens).[6,7] The majority of class I food allergens are heat stable and resistant to degradation or proteolytic digestion, whereas class II food allergens are usually easily degradable.[8] Stable class I food allergens have the potential to induce severe reactions, whereas easily degradable class II food allergens tend to induce milder reactions often limited to oral allergy symptoms.[6,8,9] Another characteristic of food allergens is the occurrence of sequential (linear), as well as conformational (discontinuous), IgE epitopes. Sequential epitopes have been suggested to be more important in class I food allergy, whereas conformational epitopes have been suggested to be more relevant in class II food allergy.[10]

Accurate diagnosis of food allergy and appropriate treatment options depend on the verification of functionally relevant, allergen-specific IgE antibodies (sIgEs), as well as on the identification of the responsible allergenic molecule or molecules. Today, a variety of *in vivo* and *in vitro* test systems are available to investigate sIgEs as biomarkers for allergy specification.[11] However, a positive sIgE test result merely identifies sensitization to a particular allergen and does not permit definitive differentiation between clinically relevant IgE reactivity (ie, reactivity that is capable to cross-link FcεRI receptors) and IgE reactivity not accompanied by clinical symptoms (ie, reactivity without an effector cell response).[11-13] As a consequence, the clinical interpretation of sIgE test results with food extracts is often impeded by clinically irrelevant food-food or pollen-food cross-reactive IgE antibodies, leading to positive test results in subjects without

From [a]the Department of Allergology, Paul-Ehrlich-Institut, Langen, and [b]the Allergy Unit, Department of Dermatology, University Hospital Zurich.

Disclosure of potential conflict of interest: S. Vieths has received an honorarium from Phadia and the Food Allergy Resource and Research Program; is associated with the Institute for Product Quality; has received research support from the European Union, the German Research Society, the Research Fund for the German Food Industry, Monsanto Co, and the European Directorate for the Quality of Medicines and Health Care; and has served as a member of the European Academy of Allergy and Clinical Immunology, the International Union of Immunological Studies, the European Agency for the Evaluation of Medical Products, ILSI Health and Environment Health Institute, the Protein Allergenicity Technical Committee, CEN, and Deutsche Gesellschaft für Allergie und Klinische Immunologie. The rest of the authors have declared that they have no conflict of interest.

Received for publication February 26, 2008; revised April 8, 2008; accepted for publication April 10, 2008.

Available online May 12, 2008.

Reprint requests: Stefan Vieths, PhD, Paul-Ehrlich-Institut, Department of Allergology, Paul-Ehrlich-Strasse 51-59, D-63225 Langen, Germany. E-mail: viest@pei.de.

0091-6749/$34.00

doi:10.1016/j.jaci.2008.04.008

clinical food allergy.[14,15] A further problem is that commercially available food extracts are often not standardized, and the content of functional allergenic molecules varies based on the nature and quality of the food, the extraction procedure, and storage conditions.[16-20]

Double-blind, placebo-controlled food challenges overcome the problem of determining the clinical relevance of sIgE reactivity, which is why they are still the gold standard in food allergy diagnostics, against which all other approaches should be verified.[11,13,21] However, *in vivo* provocation tests with food allergens carry the risk of inducing severe allergic reactions.[11,22] Therefore functional sIgE tests to detect basophil activation *in vitro* by using either patients' cells directly or serum IgE coated to a basophil cell line have been suggested as surrogates.[23,24]

Progress in biochemistry and molecular biology allowed for the identification, cloning, and recombinant production of allergenic proteins, as well as the synthesis of IgE epitope–emulating peptides of a number of food allergens.[10,16,25] These new measures enabled component-resolved diagnostics of food allergy by detecting and quantifying IgE antibodies specific to a protein or even a sequential epitope. Component-resolved diagnosis revealed individual sensitization patterns to (1) different proteins of an allergenic food, (2) homologous proteins in different foods, and (3) different epitopes of a single allergenic molecule.[26-31] Nevertheless, cumulative analyses of these results also revealed the presence of common sensitization patterns to major allergenic molecules of a single food, as well as to immunodominant IgE epitopes in a single allergenic molecule.[26,27,32,33] These findings encourage the use of panels of purified native or recombinant allergenic molecules and the use of synthetic sequential epitopes for an elaborate molecular analysis of sensitization patterns. This promises refined diagnosis, risk assessment, and prediction in food allergy. The present review aims at providing a general conspectus on the basis and current effect of diagnostic IgE-binding tests with recombinant food allergens or the respective sequential epitopes.

IMMUNOBLOT ANALYSES ALLOW FOR THE IDENTIFICATION OF TARGET MOLECULES

Immunoblot analysis of sIgE reactivity to food extracts with labeled anti-human IgE antibodies after gel electrophoresis and Western blotting of the allergenic protein source first enabled (1) the identification and discrimination of allergenic molecules from a single source and (2) the detection of individual sensitization patterns to specific allergenic molecules in different but cross-reactive sources.[34] In many cases overall sensitization to immunodominant proteins, as well as individual sensitization to minor allergenic proteins, was observed.[35-39] Nevertheless, the pattern of sIgE reactivity can be highly disparate among patients with allergy to the same food.[39,40]

Immunodominant proteins with identical or highly similar molecular masses were detected in extracts of different sources, indicating food-pollen[41] or food-food cross-reactivity.[35,36,42,43] Preabsorption of patient serum with purified native or recombinant allergenic molecules before immunoblot experiments confirmed the expression of cross-reactive sIgEs, which are capable of binding proteins from different sources with conserved binding sides and sufficient sequential, as well as structural, homology.[44] Accordingly, immunoblot inhibition studies demonstrated attenuation of sIgE reactivity to immunodominant proteins of crude allergen extracts, as well as to purified allergens, by means of preabsorption of the sera with purified proteins from a homologous source.[36,37,45-47] Reciprocal IgE antibody neutralization experiments verified the concept of pollinosis as a trigger of food allergy in terms of secondary cross-reactivity of pollen-specific IgEs to class II food allergens.[48] Accordingly, recombinant Bet v 1, the major birch pollen allergen, was shown to act as a potent inhibitor of IgE cross-reactivity to the homologous proteins from apple, carrot, celery, hazelnut, and peach,[45,47] whereas homologous Api g 1, Mal d 1, and Dau c 1 from celery, apple, and carrot were inefficient in inhibiting IgE reactivity to Bet v 1.[45] This can be explained by the "polyclonal" situation of the immune response, in which a number of different IgE antibodies recognize the primary antigen but only a few of them react with the secondary antigen.

Immunoblot analyses are also useful in evaluating the allergen-specific influence on the course of food allergy (eg, regarding the effect of individual sIgE sensitization patterns to different allergenic molecules of a single source). As an example, immunoblot analyses elucidated why allergy to certain plant foods manifests with relatively mild clinical conditions in patients with birch pollen allergy from central Europe,[6,9,14] whereas the same comestibles have the capacity to provoke severe systemic reactions in patients without (birch) pollen allergy from the Mediterranean area.[6,49] It could be demonstrated that Bet v 1–homologous class II food allergens are responsible for immunodominant sIgE reactivity in patients with birch pollen allergy, whereas sera of patients without (birch) pollen allergy frequently react to class I food allergenic lipid transfer proteins of these comestibles.[28,50-52]

Taken together, immunoblot experiments are an essential basis for the development of *in vivo*, as well as *in vitro*, sIgE test systems with purified allergens. However, an immunoblot detection of sIgEs is not a proof of clinical relevance because a single IgE epitope is sufficient for the *in vitro* reactivity.

SKIN TESTS WITH PURIFIED ALLERGENIC MOLECULES

The easiest approach in establishing whether a patient possesses sIgEs is a skin prick test (SPT) with commercially prepared allergen extracts.[53] The diagnostic potential of skin tests depends first of all on the quality of the test agents. Unfortunately, quality and composition of the available, mostly nonstandardized food extracts is highly variable. One might speculate that this is especially true for labile plant food allergens. However, skin tests with fresh extracts or prick-by-prick approaches with fresh food frequently revealed higher assay sensitivity in comparison with tests with commercial extracts for all classes of food allergens.[54-57] Nevertheless, skin tests with commercial food extracts of class I food allergens are usually characterized by high sensitivity and negative predictive values of more than 90%, whereas their specificity is generally poor, and a positive test has, on average, a 50% positive predictive value.[58] In contrast, skin tests with commercial food extracts of class II food allergens can also be vitiated by low sensitivity (eg, because of the low proteolytic stability of the allergenic components).[28,59-61]

Just as with *in vitro* sIgE tests (see below), diagnostic skin tests are further hampered by the fact that positive test results are frequently seen in a considerable proportion of individuals without adverse reactions to the respective food.[54,62-66] The significance of a positive test result with a food extract is mainly deteriorated