

Microarray of allergenic component-based diagnosis in food allergy

Maria L. Sanz^a, Ana B. Blázquez^b and Blanca E. Garcia^c

^aDepartamento de Alergología e Inmunología Clínica, Clínica Universidad de Navarra, Pamplona, ^bFundación IMABIS, Laboratorio de Investigación, Málaga and ^cServicio de Alergología, Complejo Hospitalario de Navarra, Pamplona, Spain

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Purpose of review

The determination of specific IgE (sIgE) against allergenic components fixed in a solid support that is provided as a microarray of high capacity and allows a more precise evaluation in the food allergy diagnosis. In this review, we will analyze the results obtained to date with this technology applied to the component-based diagnosis of food allergy.

Recent findings

Microarrays of proteins or glycoproteins allow us to know the profile of sensitization of a patient with food allergy. At present, a commercially available technique exists which allows sIgE to be detected against 103 allergenic molecules. Several laboratories worldwide have explored and optimized this technique for few allergen extracts and the results have been promising with high reliabilities and sensitivities and above all, good correlations with previous existing conventional assays.

Summary

In recent years, as a result of advances in molecular biology, together with the development of new technologies of producing high-capacity solid-phase matrices such as microarrays, the diagnosis of food allergy has improved and the basic situation of analyzing sIgE against an allergenic source has now become real the possibility of analyzing sIgE against an allergenic protein or glycoprotein. This change has not only led to a more precise diagnosis of sensitization, but can also be used to explain the different hazards of certain molecular sensitizations, crossreactivity phenomena in many cases and can even change the clinical management according to the information provided. Further studies are clearly needed to evaluate more precisely the scope of this new technique.

Keywords

food allergy, microarray, molecular diagnosis, peptides, specific IgE

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Introduction

Recent decades have witnessed: on the one hand, the identification, isolation and purification of a great number of allergens of different origin; and on the contrary, the application of recombinant DNA technology to the field of allergology. The biotechnical production of allergens of high purity and consistent quality, both natural and recombinant, has represented a real advance in clinical allergology [1].

The combination of these advances from molecular biology with those from the field of nanotechnology has allowed the development of new tools, among which are microarrays. Protein microarrays allow specific IgE (sIgE) against multiple molecules to be detected in the same assay thus making the so-called molecular diagnosis or component-resolved diagnosis (CRD) possible.

Clearly, this is of great relevance in the diagnosis of food allergy [2].

The first experimental microarray system for allergy diagnosis was reported in 2000 [3]. Initially, this technique was performed with a limited number of allergens obtained from whole allergenic sources [3–5] and proteins extracted from 35 different allergenic sources [6,7].

Later on, microarrays were developed to which a growing number of recombinant and purified molecules were attached. The ISAC prototype commercialized by Phadia was the first protein microarray applied to the detection of sIgE which in its current version (January 2011) is made up of 103 components (ImmunoCAP ISAC-CRD 103, Phadia, Uppsala, Sweden).

The technique was born amid great expectations as theoretically it was going to achieve the microanalysis of allergy diagnosis [8], thus giving clinicians access to detailed information regarding patients' profiles of sensitization, allowing them to sometimes predict the severity and progression of reactions and the precise indications for immunotherapy in the case of respiratory allergies and eventually other indications.

Molecular or component-resolved diagnosis

The term component-resolved diagnosis (CRD) [1] opened up a new era in the diagnostic and immunopathologic concept of allergies.

The use of an appropriate panel of purified allergens, natural or recombinant, allowed the traditional diagnostic techniques of detecting sIgE based on the use of whole extracts to be improved with regard to sensitivity, reproducibility and stability, especially in the case of plant foods with low-protein content and which possess enzyme activity which degrades the allergens [9,10].

CRD allowed individual patterns of sensitization to different proteins from the same allergenic source to be identified with the possibility of also establishing:

- (1) sensitization patterns for particular geographic areas [11,12];
- (2) sensitization to proteins associated with a greater risk of severe reactions, for example, lipid transfer proteins (LTPs) [12,13];
- (3) sensitization to homologous proteins in different foods with the possible involvement of crossreactivity phenomena in otherwise not physically related fruits [14–16].

Relevant antigens and crossreactivity

The presence of sIgE against a particular allergenic source is not always accompanied by clinical symptoms and therefore clinically relevant in food allergy. One of the common causes of these false positives is the existence of crossreactivity between homologous allergenic components present in different allergenic sources, some of which are unrelated taxonomically.

The crossreactivity between similar molecular structures present in food and aeroallergens gives rise to clinical phenomena or syndromes such as the latex-fruit syndrome, whose molecular basis is the chitinases (Hev b 2), as well as other pan allergens such as profilins (Hev b 8) and patatins (Hev b 7), the crossreactivity between Rosaceae and birch and grass pollen usually due to PR-10 and profilins respectively, the dust mite–shrimp–cockroach syndrome in which tropomyosin is involved,

chicken–egg syndrome due to livetins, pig–cat syndrome and other crossreactivity disorders between meats and epithelia with the involvement of serum albumin, among others. Furthermore, sensitization to food allergens from phylogenetically well preserved families such as storage proteins (2S albumins, vicilins and legumins) and LTPs is the cause of immunologic crossreactivity between foods (nuts, legumes and/or seeds in the case of storage proteins; Rosaceae, other fruits, vegetables and plant foods in general in the case on LTPs) with varying clinical repercussions. In any case, knowledge of the individual sensitization profile of a patient allows potential crossreactivity syndromes to be analyzed [1] which in turn allows a patient's tolerance to be tested and preventive advice to be given.

Risk of severe reactions in food allergy

An important body of evidence exists with regard to the association between certain clinical manifestations and certain patterns of recognition [12,17] in that sensitization to different allergenic components from the same source leads to different types of intensity in the response. A good example of this is the different recognition patterns of wheat components [18] in patients with baker's asthma, food allergy to wheat components, allergy to wheat pollen and anaphylaxis induced by exercise dependent on the ingestion of cereals. Furthermore, sensitization to labile molecules such as the PR-10 proteins and to profilins is related to local symptoms after the ingestion of raw vegetables, whereas sensitization to allergens more stable to physical and chemical processes such as LTPs, chitinases, vicilins, 2S albumins, legumins and 5- ω -glutadin is more likely to cause systemic reactions even after the ingestion of processed foods.

Epitopes providing information on the persistence of food allergy

A further step in the diagnostic evolution is the identification of the epitopes of an allergenic molecule against which sensitization has occurred [19]. Lin and Sampson [20] described the potential role of sequential IgE-binding epitopes as biomarkers for characterizing various phenotypes of food allergy, using microarray technology. They considered it to be a robust technique applicable to the epitope mapping of food allergens and suggested that the microarray might be even more sensitive than SPOTS membrane technology [19].

Sera of patients with persistent food allergy to egg recognize sequential epitopes and have higher levels of sIgE than the serum of patients who achieve tolerance [21].

In milk allergy, it has been confirmed that IgE to distinct allergenic epitopes of cow's milk proteins can be used as a

marker of persistent allergy. The successful union of these peptides in a matrix should enable large prospective studies to be conducted and future diagnostic approaches to be simplified [22].

In infants with cow's milk allergy, early recovery was observed associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes [23]. Patients with milk allergy recognize a greater range of epitopes bound to IgE in comparison with those who have overcome their allergy and who in this respect are similar to patients with tolerance. The binding of a greater number of peptides to IgE is associated with more severe reactions in the course of challenge tests [24].

Studies validating microarray technology using home work or commercially available technology (ISAC) in food allergy

When this technology has been compared to conventional solid-phase assays for detecting IgE, appropriate correlations have been observed [4,7,25–28], although for some allergens the sensitivity of the microarray technique can be lower [28] as a result of the lack of glucidic modification of proteins produced in the bacterial systems which play an important role in the binding with IgE [29] or due to the incompleteness of the panel of possible allergenic components of sensitization to an allergenic source. Ott *et al.* [30**] analyzed 130 children with suspected IgE-mediated food allergy with a challenge test with cow's milk and/or egg, skin tests and sIgE. They applied a Microarray (ISAC CRD-51, VBC Genomics Bioscience Research, Vienna, Austria) which included ovomucoid, ovalbumin, lysozyme, α -lactalbumin, β -lactoglobulin and caseins. The positive predictive values of ISAC CRD-51 were 77% for milk and 100% for egg when all the components were analyzed as one set. The authors concluded that the milk and egg components of this microarray yield results equivalent to those obtained with skin tests and/or sIgE, without any single component discriminating between and irrelevant and genuine sensitization.

Furthermore, Gaudin *et al.* [27], using their own microarray, analyzed the proteins most frequently involved in children's sensitization to milk and identified different recognition profiles between those with and without clinical manifestations and found that bovine lactoferrin is a major antigen in milk allergy.

It has been reported that in children with suspected allergy to milk and/or egg ISAC CRD-89 (Phadia) is capable of predicting a positive challenge test and could be used as a second-level assay, if the ImmunoCAP sIgE is less than 95% clinical decision points, thus reducing the need for challenge tests [31].

Children with shrimp allergy showed greater shrimp-sIgE antibody levels and more intense binding to shrimp peptides of tropomyosin, arginine kinase, myosin light chain, and sarcoplasmic calcium-binding protein and greater epitope diversity than adults [32], which suggests that shrimp sensitization decreases with age.

Constantin *et al.* [18] also developed a microarray of allergenic molecules and concluded that it will be possible to design serological tests based on microarrayed recombinant wheat seed and grass pollen allergens for the discrimination of baker's asthma, wheat-induced food allergy and grass pollen allergy.

Differential epitopes have been identified in cases of exercise-induced anaphylaxis [33]. Battais *et al.* [34] identified certain IgE-binding epitopes on gliadins related to the presence of the food allergy to wheat flour.

Different studies undertaken with patients with peanut allergy [35,36] have shown that sensitization to certain allergenic protein of peanut as determined by microarrays may be related to the severity of clinical symptoms. Patients with a history of more severe multisystemic reactions recognize a greater number of epitopes of Ara h 1, Ara h 2 and Ara h 3 and a correlation exists between the range of Ara h 1, Ara h 2, Ara h 3 epitopes recognized by a patient's IgE and the severity of the clinical picture [37]. However, Flinterman *et al.* [37] found no group of peptides associated with severity. There seem to be differences in the patterns of sensitization of children who are allergic or tolerant to peanut with the Ara h 2 component being the most important in the prediction of clinical allergy to peanut [38]. Furthermore, geographical differences exist in patients with peanut allergy and peach allergy in the Mediterranean area [39] studied with a microarray including Ara h 9, Ara h 1, Ara h 2, and Ara h 3, where it was found that Ara h 9 (LTP) seems to play an important role in allergy to peanut.

Several IgE-binding sequential epitopes of Len c 1 have been identified [40]. Epitope diversity is correlated with IgE levels, pointing to a more polyclonal IgE response.

Act d 1 can also be considered a marker allergen for genuine sensitization to kiwifruit and it has been demonstrated that a component-based kiwifruit allergen microarray would improve the prognostic value of in-vitro diagnostic tests [41]. However, the ISAC (Phadia) offers poor sensitivity for Act d 8 and Dau c 1 (Bet v 1 – homologous in kiwi and carrot) in patients with a history of oral allergy syndrome (OAS) [42].

Nevertheless, Ebo *et al.* [43] found that the microarray was a reliable instrument in the diagnosis of apple-mediated OAS in birch pollen allergy. However, for

discriminating between sensitization and a real allergy, microarrayed rMal d 1 offered no advantage over conventional quantification of rMal d 1 sIgE.

As for the association between latex and food allergy, Radauer *et al.* [44] studied 16 408 allergic patients' sera for IgE binding to hevein, latex chitinase, and wheat germ agglutinin using an allergen microarray and detected 58% of the cases positive to hevein. Hevein-like domains (HLDs) of latex, banana and avocado chitinases were positive in 36, 34 and 15% of the sera, respectively. In contrast, only one of 20 banana-allergic patients without latex allergy was sensitized to chitinase HLDs.

Verweij *et al.* [45] studying infants under 1 year of age with atopic dermatitis and using an ISAC microarrays with 103 components (Phadia) found that the majority of patients were sensitized to Cor a 9, an LTP potentially dangerous hazelnut component. The CRD by microarray involving quantification of sIgE to Cor a 1, Cor a 8, and Cor a 9 detected sensitization to hazelnut in only 60% of patients identified as hazelnut-sensitized by a traditional hazelnut sIgE assay. The reason for this is most likely the absence of components such as Cor a 11 in this ISAC version.

Parallel determination of several AC isotypes (immunoglobulins A, M, G and E)

The parallel determination of different antibody isotypes (IgA, IgM, IgG and IgE) using microarrays seems to offer promising results even when attachment to the microarray is achieved using whole food extracts. It is possible to discriminate between atopic patients with positive challenge tests and nonatopic controls and the determination of sIgE using this technique shows a high correlation with results obtained using ImmunoCAP (Phadia) [46].

Noh *et al.* [47^{••}] performed allergen-sIgE/IgG4 measurements using a protein microarray of their own making in atopic dermatitis patients with a history of food-specific hypersensitivity and increased food-specific IgE levels. A double-blinded placebo-controlled food challenge test (DBPCFC) was performed for the diagnosis of allergy to milk, egg white, wheat, and soybean. Mean IgE/IgG4 levels in DBPCFC (+) patients were higher than those in DBPCFC (–) patients in all the food items studied. The specific IgE/IgG4 ratio using the microarrays was able to indicate the severity and progression of the atopic dermatitis.

Detection of IgE epitopes in food allergens using a combination of two techniques: microarrays and basophil activation

A novel and promising alternative in the diagnosis using microarrays is the proposal of coupling microarray tech-

nology with exposure to effector blood cells carrying IgE. This could represent a biological in-vitro assay that is very close to the mechanisms that actually operate *in vivo*. In this sense, Lin *et al.* [48^{••}] observed the activation of effector cells induced by epitopes from food allergens previously fixed in a microarray.

Similarly, Hochwallner *et al.* [49] designed a proprietary microarray by fixing cow's milk allergens to subsequently determine IgE reactivity profiles in the serum of 78 cow's milk-sensitized individuals. They attached the IgE of the patients' serum to rat basophil leukemia cells expressing the α -chain of the human receptor Fc ϵ RI and assessed the frequency of IgE recognition and basophil activation in response to the proteins attached to the microarray. This assay was able to distinguish individuals with or without mild clinical reactions from those with severe systemic or gastrointestinal symptoms as well as persons who grew out of cow's milk allergy from those who did not.

Conclusion

Molecular diagnosis by microarray technology opens a new era in food allergy diagnosis and in the management of the allergic diseases allowing a more precise identification of the causal agent, helping to distinguish a real allergy from a hypersensitivity; prediction of natural history or persistence; prediction of severity of the clinical symptomatology; an accurate indication of antigen-specific immunotherapy; a more effective monitoring of the allergic diseases; to avoid in some cases performance of challenge tests; and to be considered an auxiliary tests to guide the challenge test when the diagnosis by other techniques is not conclusive.

In the diagnosis of food allergy, this technology also allows allergists to determine the patterns of sensitization presented by a particular patient and the differences which may exist with those typical of a given geographical area and to detect sensitization to molecules responsible for crossreactivity syndromes.

Further validation studies are required given that studies do not always provide sufficient information to allow a reliable interpretation of the relevance of the sensitizations detected using this technique [50]. In line with this, studies with sufficient statistical power to validate the technique need to be conducted [16]. Furthermore, as it has been pointed out by Salcedo and Diaz-Perales [13], the technique suffers from certain limitations such as the composition of the panel of allergens, which may not be the most appropriate for some geographical areas. In fact, further studies are needed to customize the technology to the different geographic areas of the world where both environmental plant-derived allergens (pollens) and food have big differences.

Furthermore, there is a real need to evaluate the sensitivity, specificity and cut-off points for each allergen in series of truly allergic patients and in controls for each protein included in the microarray in order to be able to situate this promising tool in its corresponding place in the diagnosis of allergy in general and in that of food allergy in particular. We must not forget that all in-vitro tests should be evaluated together with the clinical history, because allergen sensitization does not necessarily imply clinical responsiveness. Another important fact to be considered is that although sensitizations can be described in large scale with a large number of allergens, patients can be tolerant in a substantial part. This requires undertaking detailed clinical evaluations and studying what are the additional factors associated with clinical response or tolerance.

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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