



The added value of allergen microarray technique to the management of poly-sensitized allergic patients

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Purpose of review

To evaluate the relevance of results obtained using allergen microarray technique for the description of the IgE repertoire in allergic patients.

Recent findings

Allergen microarray was introduced at the beginning of the last decade. Since then, an increasing number of allergens have been identified, correspondingly increasing the accuracy of the description of immunoglobulin (Ig)E repertoire. In the last 2 years, a large number of articles were published that accurately described not only the general features of this technique, but also the use of allergen microarray in specific situations.

Summary

The recent availability of highly purified or recombinant allergen components has deeply modified the laboratory approach to allergy diagnosis that, now, it cannot be limited to the detection of IgE specific to extractive allergens. Indeed, these contain both specific components (i.e. molecules strictly associated to that allergen source) and pan-allergen or cross-reacting allergens (i.e. molecules that are present in different similar allergen sources or that are present in highly homologous structures in different species). Newer techniques such as recombinant allergen testing and allergen microarray allow a more detailed evaluation of IgE responses. Future research is needed to more clearly define their role in clinical practice.

Keywords

allergen, component, evidence-based medicine, IgE repertoire, immune-solid-phase allergen chip, microarray

INTRODUCTION

The availability of new reagents for analysis of allergic sensitization, represented by purified natural components and recombinant molecules, has significantly modified the diagnostic approach of allergy. Indeed, an accurate dissection of the IgE repertoire is needed not only to better define the diagnosis, but also to identify the allergen source(s) required for the prescription of a specific immunotherapy. Extractive allergens are known to be constituted by a mixture of components that can be identified as 'specific' and 'nonspecific' components. Specific components are the molecules restricted to the allergen. Nonspecific components are shared with other allergen of the same family (pan-allergens) or of different families (cross-reacting allergens). Any immunotherapy attempt should ideally be carried on using specific

components. In addition, an accurate dissection of the immunoglobulin (Ig)E repertoire allows the explanation of certain complex clinical features, caused by an inhalant allergen cross-reacting with a food component. A complete and exhaustive description of the rules that condition the

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KEY POINTS

- Dissection of immunoglobulin (Ig)E repertoire is significantly improved using allergen components.
- Allergen microarray offers several added values, in particular, identification of genuine, cross-reacting and pan allergens.
- Allergen microarray has a clinical relevance when correctly positioned in the allergy diagnosis flowchart.
- Molecular allergy is linked to new approaches of system biology.

relationships between allergens was written by Sastre [1]. Since their discovery, specific IgE antibodies were captured by the immobilized allergen and the immune complex was efficiently revealed using a tracer-labelled anti-IgE. The large amount of allergen assured that a wide range of IgE concentrations were detected and that the effects of any interfering substances, such as specific IgG that could compete with IgE, were irrelevant. In 2002, a different approach was attempted: small amounts of homogeneous components were added to a glass slide in order to build an array. Microarray proved effective because many components were in a small surface, few drops of serum were needed and fewer but purified components were spotted. The availability of different components resulted in the 'component-resolved diagnosis' (CRD) approach [2–6]. More recently, allergen microarrays were commercially distributed and the name 'immune solid-phase allergen chip' (ISAC) started to circulate in allergy congresses. At present, its contribution to the accuracy of allergy diagnosis is more and more significant. In this work, we revise the literature on allergen microarrays in the very recent period and try to define how this technology can be integrated with other diagnostic tools to improve allergy diagnosis. Thus, the best articles were considered and other related articles were only reported to support the conclusions.

THE LABORATORY TECHNIQUE

In a recent review, it was indicated that the main application of allergen microarrays is IgE profiling of patients with broad sensitization [7], but it was also argued that, on a molar basis, the allergen could be a limit, for the potential interference between IgE and other isotypes, principally IgG. Although reported by others that the presence of limited amounts of allergen could interfere with IgE in patients with food allergy or subjected to immunotherapy [8], in the context of allergic disease, IgG does not have

IgE-competing capacity, due to its different affinity: indeed, affinity of IgE for the allergens may be among the strongest biologically relevant forms of binding, being in the pM range versus the nM range for IgG [9]. Along this line, it had already established that the risk of interference was small: an excess of antigen has been regarded as a rule for increasing the sensitivity of the in-vitro tests, but it has been reported that such allergen-specific IgE levels are not always correlated to clinical and biological sensitivity [9]. The assumption that a test involving a small amount of allergen does not necessarily imply a disadvantage has been reinforced by the excellent correlations observed between microarray testing and a routine standard test involving excess allergen [4]. In addition, in recent work [10[¶]], using IgG removal, the absence of any apparent effect of IgG to IgE binding was also experimentally demonstrated. A novel approach was described in 2010, using a different immune-sorbent substrate [11]. Indeed, it was found that copoly(DMA-NAS-MAPS)-coated glass slides yielded results in allergen microarrays overlapping or higher than previously published data generated with a commercial microarray.

WHEN SHOULD MICROARRAY BE CONSIDERED?

To define when allergen microarrays may be indicated in the diagnostic flowchart, several parameters should be considered. In particular, the cost of allergen microarrays: this must be weighed against the amount of information obtained, the small sample volume required and particularly the probable benefit of determining in an inaccurate way the IgE profiling in poly-sensitized patients [12]. All these considerations allowed concluding that allergy diagnostic could change from current skin prick test (SPT) and sIgE to IgE multiplex integrated by SPT and sIgE if needed [13]. In this context, other authors [14] recognize that the approach has a relevant value providing information that largely exceeds the output from current sIgE assays. Nevertheless, at present, it should be considered a second-third level tool rather than a first-line choice, because allergen components do not always represent the whole extractive allergen and often, microarray testing sensitivity is sometimes lower than that of both extractive and recombinant allergens used as single reagents.

COMPARISON BETWEEN ALLERGEN MICROARRAYS AND OTHER REFERENCE TESTS

In a study conducted using 321 patients and 92 controls [10[¶]], the reproducibility of allergen

microarrays was good and the positive percentage agreement was 75–100% for sIgE levels more than 1 kUA/l. For samples with sIgE levels less than 0.1 kUA/l, the negative percentage agreement was 90–100%. Notably, 58% of respiratory allergy patients had IgE to food-specific proteins and 52% resulted sensitized to cross-reactive pan-allergens. For the first time, the global added values of allergen microarrays were evaluated and included detection of IgE specific for tropomyosins, profilins, parvalbumins, storage proteins, lipid-transfer proteins, calcium-binding proteins, pathogenesis-related-10 (PR-10) and so on. Eighty-six patients were evaluated using specific IgE for recombinant allergens and allergen microarrays [15[¶]]. For IgE positives, the concordance was 78.65% and for negatives, the concordance was 93.57%. Standard IgE testing and CRD were also compared and this approach modified the conventional diagnosis in 30% of cases [16]. Finally, allergen microarray was compared with the whole-extract ImmunoCAP in grass and cypress pollen allergy [17], concluding that the cut-off point for each allergen should be properly applied for both techniques.

CLINICAL USE OF ALLERGEN MICROARRAYS

Allergen microarray was also tested in different and specific clinical situations, even though a general study evaluating the whole capacities of allergen microarrays to define what are the specific added values in the real life of allergy practice is still missing. Nevertheless, all these contributes have special values, because, using the microarray approach, it was possible to better define several different sensitizations. Some works were performed by using the commercially available allergen microarray, whereas others were performed by using customized or experimental versions of the assay. Allergen microarray was used [18] in a cohort of patients with Atopic Dermatitis, suffering also for other allergic symptoms (half with respiratory symptoms, 25% with contact allergy and three with severe food allergy). A mean of 21 sensitizations per patient was observed: more than 50% of the patients were sensitized by nine plant and eight nonplant proteins, frequencies that were different from those observed in different age intervals on unselected sensitized patients [19[¶]].

As far as the wide field of food allergy regards, a recent issue of *Current Opinion in Allergy and Clinical Immunology* extensively treated the argument [20–26]. In different experimental conditions, a prototype containing 350 food extracts was developed. IgG specificity had a good correlation with

IgA, a worse correlation with IgM and it was very bad with IgE. The simultaneous profile for IgA, IgM, IgG and IgE allowed a good discrimination between atopic and nonatopic individuals [27].

Other studies were performed by using purified or recombinant components in food allergy patients. For example, starting from the evidence that in-vitro and in-vivo tests on kiwifruit extracts frequently fail to detect specific IgE, nine kiwifruit components were studied in a custom-modified allergen microarray. The diagnostic sensitivity was 66%, better than ImmunoCAP (47%) and approached that of the recombinant ImmunoCAP analysis (77%) [28]. Furthermore, comparing the specific IgE profiles of 30 patients with a positive double-blind placebo-controlled food challenge to kiwifruit, a significant correlation in the case of Act d1, Act d2 and rAct d 9 [28] was observed. Similar results were also obtained by others [29].

For milk allergy, using a customized allergen microarray, it was found that 32.7% patients were monosensitized. The other 67.3% reacted with at least two allergens. No clear differences regarding monosensitization or oligosensitization was associated to age even if it was observed that the higher cow's milk-specific IgE levels were associated with oligosensitization [30].

In Hen Egg allergy, it was found [31] that Gal d1 positivity is a good predictor. Gal d1-positive children have a high frequency of Hen Egg allergy, whereas Gal d1 negatives tolerate boiled egg. Nevertheless, according to this study, allergen microarray showed a low sensitivity.

For Latex allergy, in a wide study [32], 102 adults were tested by using eight recombinant Hevea b. components. rHev b5 and rHev b6.02 were the most relevant components, eliciting positive sIgE results in 44 and 69% of patients, respectively. sIgE antibodies against rHev b11 could only be detected in patients also sensitized to rHev b6.02.

An interesting field is the cross-reactivity causing food allergy in patients with respiratory allergen sensitization [33[¶]]. The high frequency of *Parietaria* pollinosis in the Mediterranean area was associated with pistachio nut sensitization, the Pistachio–Pellitory association. In contrast, *Artemisia* sensitization has been implicated in several pollen-food syndromes mediated by a very large number of vegetables.

Epitope analysis may better clarify the allergy to food components. The IgE binding to synthetic peptides representing four allergens of a shrimp were analyzed [34^{¶¶}]. Positive challenges (46% of 37 studied) showed more intense binding to shrimp peptides than those with negative challenges. Diagnostic efficiency was higher for epitopes from

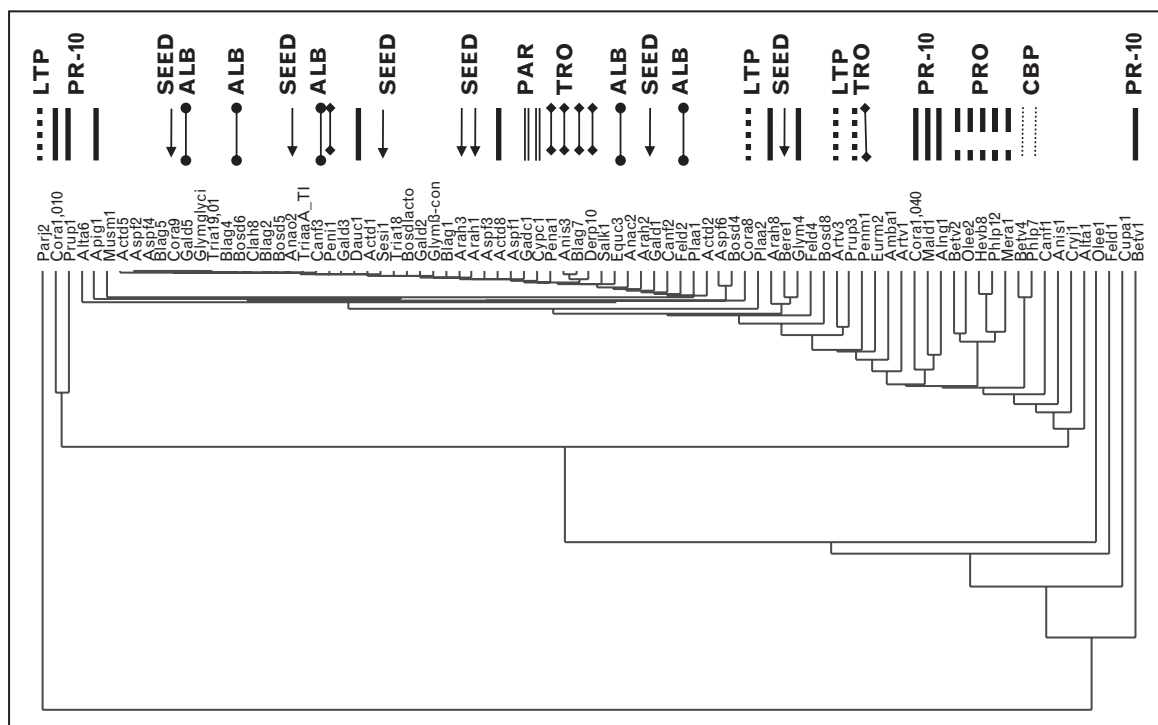


FIGURE 1. Cluster analysis of results obtained using ISAC in poly-sensitized patients with respiratory allergy. Results obtained in the course of the study of Melioli *et al.* [10⁹] were evaluated using cluster analysis after removal of the main genuine components. As also described in the text, some families of pan or cross-reacting allergens clustered together, whereas others (such as the PR-10s) had a very heterogenous behaviour. This analysis may have a certain impact on the concept of sensitization to component families. ISAC, immune-solid-phase allergen chip.

The modification of allergen component sensitization from newborns to adults was followed up using allergen microarrays and/or recombinant molecules. In a very well conducted study [37²²], it was clearly demonstrated that IgE sensitization profiles were highly heterogeneous: among the 176 of 200 children with IgE to *Phleum pratense*,

Microarray technique, for its characteristics, is highly suggestive of a system biology approach. System biology is a biology-based inter-disciplinary study field that focuses on complex interactions in biological systems. System biology uses a holistic approach: thus, biological systems are viewed as wholes, not as collections of parts instead of reductionist approach, which provides that biological systems are reduced to the interactions of their parts. Indeed, a systemic approach is virtually intrinsic, being molecular allergy constituted by different sceneries – inhalants, foods etc. – and having in these sceneries a large number of strict interactions, pan and cross-reacting allergens. In a very wide group of Italian patients [40], structures belonging to the same source and defined as ‘genuine’ allergens clustered together (Phl p1, Phl p2, Phl p5, and Phl p6, or Par j1 and Par j2, or Derp1, Der p2, Der f1 and Der f2). Pan allergens (i.e. polcalcins, tropomyosins, profilins and nsLTP) were also clustered with other members of the same families. We also observed that profilins, tropomyosins and polcalcins were identified as clusters, nsLTP only partially, but the Betv1 family and serum albumins were not (Fig. 1). It was also shown that hierarchy of cross-reactivity may play an important role [41[■]]. Positive sera to Bet v1 (22.3%) were more frequent in Northern Europe than in the south (9.1%) and these ‘southern’ positivities could not be genuine because birch pollen is rare in South-Europe [36]. Thus, other homologous components probably sensitize the patients. Unfortunately, the molecular analysis of sequence homology cannot clearly explain the finding, suggesting a very complex component hierarchy [42[■]].

CONCLUSION

That allergen microarray technology had offered a very large amount of information in the last years is indubitable. Not only a more complete description of the IgE repertoire in different patients, ages and diseases is now available, but also the impact of this novel approach to the solution of complex problems of allergy sensitization, even in terms of system biology approach, has been proposed. Nevertheless, from the allergist point of view, the position of allergen microarrays in the flowchart of allergy diagnosis should be better defined. Indeed, despite the indication that allergen microarray, for its characteristics, could be used as first-line assay [13], other evidence, including costs and diffusion of the technology [12], indicate that its position is following extract-based SPT and sIgE, in an accurately selected population of poly-sensitized patients [7], particularly with a combined inhalant

and food allergy. Nevertheless, further clinical and laboratory studies are still needed to better define how the added values of this approach can be used in clinical allergy.

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Conflicts of interest

The authors G.M., S.B. and G.W.C. are members of the Italian Board for ISAC.

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the article.

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