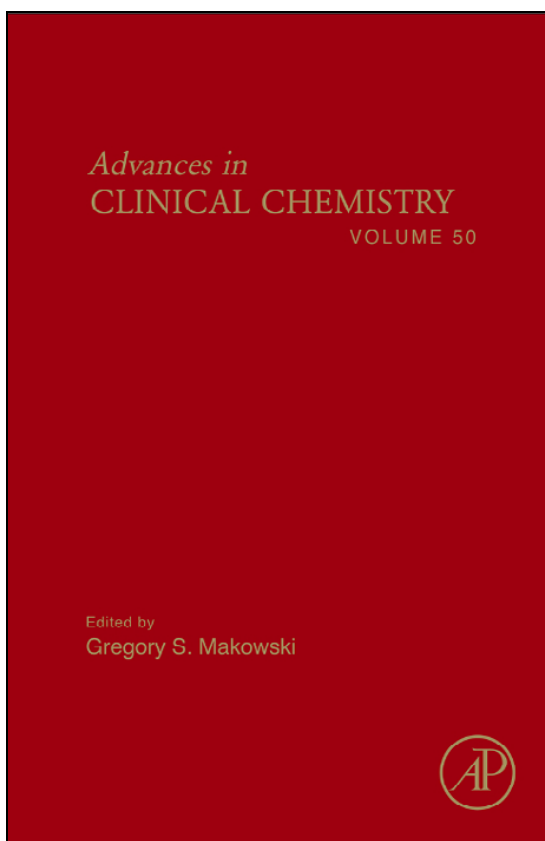


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COMPONENT-RESOLVED ALLERGY DIAGNOSIS BY MICROARRAY: POTENTIAL, PITFALLS, AND PROSPECTS

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1. Abstract

Diagnosis of IgE-mediated allergies is not always straightforward, as traditional tests can yield equivocal or negative results and provocation tests are hampered by several practical and ethical limitations. During the last decades two new *in vitro* techniques have entered the field of allergy diagnosis, that is, flow-assisted analysis of allergen-specific activated basophils and component-resolved diagnosis (CRD). This review focuses on component-resolved allergy diagnosis by microarray that has evolved from recent advances in molecular allergology and biochip technology.

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The technique allows a comprehensive analysis of individual sensitization profiles with multiplexed purified and recombinant allergens within a single run using only a minute amount of serum, providing information that largely exceeds the output from current sIgE capturing tools. Actually, multiplexing allows identification of diagnostic patterns that may facilitate the formulation of diagnostic algorithms. Although CRD by microarray sounds promising, the diagnostic performance requires further intensive assessment before it can enter mainstream application. In our opinion, the technique should currently be considered a complementary diagnostic tool rather than a first-line choice.

2. Introduction

IgE-mediated allergies constitute an important health problem and correct diagnosis is an absolute prerequisite for appropriate and potentially life-saving preventive measures or initiation of specific immunotherapy (SIT). Generally, physicians rely upon a well-taken history, quantification of allergen-specific IgE (sIgE), and skin tests to confirm their clinical diagnosis. However, none of these tests demonstrates an absolute predictive value and diagnosis is not always straightforward as a positive result merely reflects sensitization to a particular allergen without definite differentiation between clinically relevant and irrelevant IgE results [1, 2]. Therefore, challenge tests are still regarded as the gold standard in the diagnosis of allergy. Unfortunately, there are multiple practical (long duration, blinding) and ethical (e.g., severe anaphylaxis) limitations that hamper the use of challenge tests in daily clinical practice. Consequently, a consensus exists regarding the need for additional and more reliable *in vitro* tests which can improve diagnosis of IgE-mediated allergy and eventually replace or reduce the need for potentially dangerous provocations.

This review focuses on the potential, shortcomings, and prospects of an *in vitro* test that has recently entered the field of clinical allergy, that is, component-resolved diagnosis (CRD) by microarray. We also summarize our current experience with this technique.

3. Historical Perspective

Since 1880 when Blackley [3] inoculated his own skin with different pollen extracts, the diagnosis of type I allergy has generally been established by provocation tests with extracts prepared from putative natural allergens. Almost a century later, shortly after the discovery of IgE antibodies [4], the radioallergosorbent test (RAST) was developed [5]. The RAST and its

derivatives are based on the detection of sIgE antibodies directed against a number of different (genuine and cross-reactive) allergen components present in the natural allergen extract. The inherent complexity, variability, and instability of natural allergens and the considerable variation between individual sensitization profiles complicate the correct interpretation of extract-based test results. For example, it has repetitively been demonstrated that ubiquitous structures such as profilins and cross-reactive carbohydrate determinants (CCD) present on glycoproteins of plants and hymenoptera venom can result in a false-positive outcome of the serologic diagnosis of allergic diseases. Sera containing antiprofilin and anti-CCD sIgE antibodies display a very broad spectrum of cross-reactivity involving pollen, latex, fruit, vegetables, and venoms extending beyond clinical relevance, as these antibodies rarely give rise to clinically relevant allergy [6–12].

Recent progress in biochemistry and molecular biology allowed the identification, cloning, and recombinant production of relevant allergen proteins and epitope-emulating peptides, enabling detection and quantification of IgE antibodies to these proteins or sequential epitopes, that is, CRD. The first example was the cloning of the major allergen Der p 1 from house dust mite (*Dermatophagoides pteronyssinus*) in the late 1980s [13]. In contrast to traditional sIgE assays, CRD does not rely upon whole extract preparations from native allergens (generally ill-defined mixtures containing allergenic as well as nonallergenic components) but on sIgE antibodies to single components, purified from natural sources or obtained by recombinant techniques. The principles, potential, and pitfalls of CRD have been detailed elsewhere [14–19]. Briefly, CRD includes specific marker allergens and substructures to investigate the genuine sensitization of patients towards a given allergen source as well as cross-reactive determinants or allergens, such as certain glycan structures or profilins, that point to cross-sensitization to several, even unrelated allergen sources which are only weakly associated with clinical allergy. These “gatekeeper” tests not only create the opportunity to establish individual sensitization profiles (which parts of an allergen are recognized by each individual patient) [20–29] but also allow improved discrimination between sensitization and genuine allergy [25, 30]. Allergy diagnosis using CRD enables an individual risk assessment of severity and possibly prediction of persistence [27, 30–33], facilitates the selection of patients for SIT, and may help in monitoring the immunological effects of a treatment [34]. In recent years, many of the relevant allergens have been characterized and produced at the molecular level, and most have been generated as purified or recombinant proteins. When using these individual molecules for the diagnosis of allergy, the number of tests necessary to provide a comprehensive diagnosis increases considerably since more than one component needs to be included in order to identify the entire repertoire of disease

relevant peptides and epitopes. For several reasons, it has become virtually impossible to analyze all individual allergen components by traditional singleplexed sIgE tests in one patient, thus giving rise to the microarray as an elegant solution for the multiplex determination of allergen-specific IgE reactivity.

4. Microarray: The Technique

The microarray technique for CRD enables sIgE testing in a multiplex format and allows the simultaneous measurement of many IgE clones [35, 36]. The major advantage lies in the potential to investigate large numbers of allergens in parallel, detecting sIgE abundance, functionality, and interaction concerning numerous allergenic determinants using only minute amounts of serum (i.e., in children). However, this “random testing” generates a high amount of not necessarily clinically relevant information. Interpretation personalized to each individual patient using history and skin prick testing is still necessary. Also adequately powered trials are required before this technique can be introduced into daily clinical practice.

The microarray is a solid-phase multiple immunoassay in which proteins are immobilized on a solid phase (Fig. 1). The technique uses nanoliter

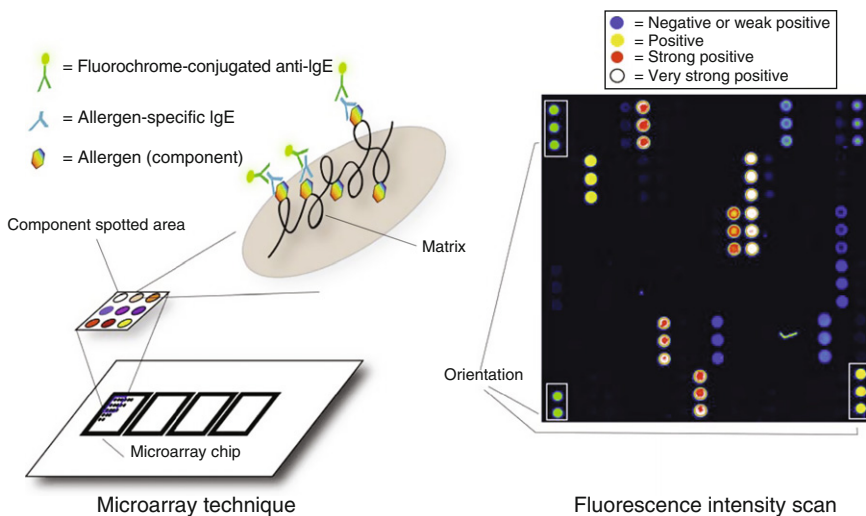


FIG. 1. Component-based allergy diagnosis by microarray: incubation of allergen chips with 20 μ l serum allows allergen-specific IgE antibodies to bind to spotted allergen components. Analysis of fluorochrome-labeled anti-IgE antibodies using a confocal laser scanner.

aliquots of purified or recombinant allergen components spotted onto micro-metric spaces of precoated glass slides in groups representing distinct allergen sources. The principles of the ISAC ImmunoCAP (ISACTM, VBC Genomics Bioscience Research, Vienna, Austria) technique used at the University of Antwerp, in which up to 103 allergens from 47 allergenic sources (spotted in triplicate) are tested in a single analysis, are described briefly [35–38], and are summarized in Fig. 1. Microarray reaction sites are incubated with a minute amount (20 μ l) of undiluted patients' serum so that allergen-specific IgE antibodies are captured by their corresponding component. Subsequently, the microarray slides are rinsed and washed with buffer solution in order to remove unbound serum sIgE. After drying, complexes of component-bound sIgE are stained with a secondary, fluorescence-labeled antihuman IgE. After a second rinsing and washing procedure, the obtained fluorescence signals are analyzed using a laser scanner. Analysis of the corresponding digitized microarray image is performed with specialized software and image information is transformed into numerical data according to a reference serum of known IgE content. Data are expressed as ISAC Standardized Units (ISU/L). The composition of the employed microarray can be found at <http://www.vbc-genomics.com> or via <http://www.phadia.com>.

5. Clinical Experience with CRD Using Microarray

Since its introduction in the field of allergy diagnosis [16], only a few papers have been published on the application of CRD testing by microarray. In the pioneer study by Hiller *et al.*, 94 purified allergen components were analyzed (78 recombinant and 16 natural) representing the most prevalent inhalant allergens such as pollen (trees, grasses, weeds), food, mites, molds, mammalian allergens (epithelia, serum proteins, saliva proteins), hymenoptera venom, insects, and natural rubber latex in sera of 20 (generally multisensitized) patients but only one healthy control individual. It was demonstrated that the clinical sensitivity was well reflected by the IgE reactivity pattern to the microarrayed allergen components from the respective sources. However, no strict association between the fluorescence intensity as measured in the microarray-based system and the magnitude of wheal reactions in the skin test was demonstrated. In a comparative study between the microarray using recombinant allergens—Phl p 1, Phl p 2, Phl p 5, and Phl p 6 of timothy grass (*Phleum pratense*) and Bet v 1 and Bet v 2 of birch (*Betula verrucosa*)—and the conventional singleplexed CAP/RAST method to detect allergen-specific serum immunoglobulin E, Jahn-Schmid and coworkers [39] demonstrated the two techniques to be highly correlated

and reliable in the diagnosis of grass and tree pollen allergy. The study by Wöhrl *et al.* [40], which enrolled 120 patients, demonstrated that CRD by microarray performs equally well in receiver-operating characteristic (ROC) curve analysis as the “reference” ImmunoCAP in diagnosing allergy to cat epithelium and pollen from birch and grasses. However, the authors observed that the microarray was slightly less sensitive for the diagnosis of house dust mite allergy and displayed a reduced sensitivity in the mugwort (*Artemisia vulgaris*) allergic patients. The authors hypothesized that the poor performance of the employed rArt v 1 could be associated with the quality of the protein preparation or the lack of carbohydrate chains, which are crucial for recognition by IgE antibodies from certain Art v 1-allergic patients [41].

Ott *et al.* [42] were the first to investigate the clinical usefulness of CRD by microarray in children with suspected allergy to cow's milk and hen's egg. This study, which involved 145 challenge tests conducted in 130 infants, concluded that allergen microarrays provide a new tool in diagnosing symptomatic cow's milk and hen's egg allergy and that the technique has performance characteristics comparable to the current diagnostic tests and may be indicated in small children in whom only small blood volumes are obtainable (e.g., capillary sampling). However, no single allergen component was found to be superior in discriminating between clinically irrelevant sensitization and genuine food allergy. It was not possible to identify a single marker allergen that significantly enhanced *in vitro* test performance. The technique was in most cases not capable of replacing double-blind, placebo-controlled food challenges. In the same issue of *Allergy*, Gadermaier *et al.* [43] demonstrated that, in their population, Art v 1 and Art v 3 were recognized by 89% of mugwort pollen-allergic patients and that CRD added to the differential diagnosis between mugwort and ragweed pollen allergy.

Finally, Constantin and coworkers [29] recently anticipated the possibility to design assays based on microarrayed recombinant wheat and grass pollen allergens to discriminate baker's asthma, wheat-induced food allergy, and grass pollen allergy. In isolated cases, CRD microarray contributed to diagnosis of pollen-associated soy allergy [44] and food-dependent exercise-induced anaphylaxis due to soybean β -conglycinin in tofu [45].

6. Experience with the Microarray at the University of Antwerp

When discriminating between sensitization and genuine allergy, traditional IgE assays have repeatedly been demonstrated to be of limited value. Therefore, in order to assess the potential of the microarray in discriminating

between clinically relevant and irrelevant sIgE antibodies, we conducted several evaluations. In a first study [37] we evaluated whether CRD by microarray could constitute a reliable instrument in the diagnosis of apple-mediated oral allergy syndrome (OAS) in birch pollen-allergic patients (Figs. 2 and 3). Although the recombinant form of the major apple allergen Mal d 1 (*Malus domestica*) was absolutely discriminative between OAS patients and healthy control individuals (Fig. 2), it offered no advantage over conventional ImmunoCAP for rMal d 1 and failed to discriminate between sensitization and real allergy [46]. However, we observed that birch pollen-allergic patients without OAS to apple display a broader sensitization to classical inhalant allergens than birch pollen-allergic patients with an apple-related OAS (Fig. 3). A second model to assess the potential of the microarray to discriminate between sensitization and allergy implied a comparative analysis of immunoglobulin E reactivity profiles in patients allergic or sensitized to natural rubber latex (Figs. 4 and 5) [38]. In all patients diagnosis of latex allergy could be documented by microarray CRD. Over three-quarters of our latex-allergic patients were sensitized to Hev b 5 and/or Hev b 6.02, both well-known major allergens from natural rubber latex

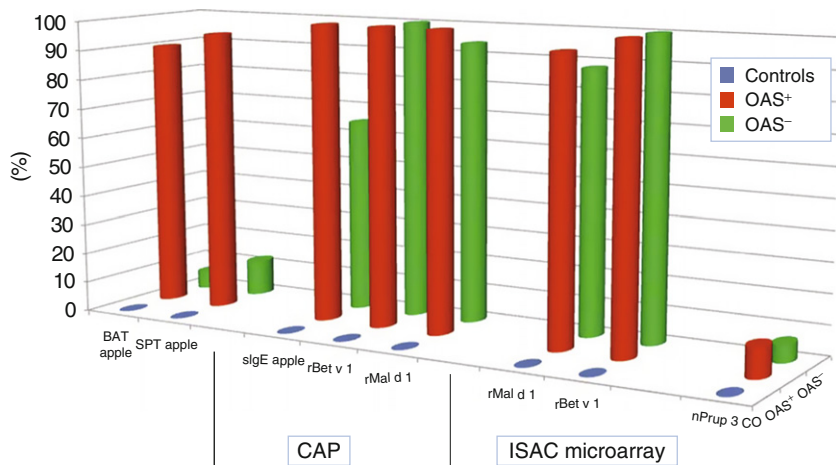


FIG. 2. Percentages of positive basophil activation tests (BAT) to apple, skin prick tests (SPT) to apple, and sIgE results by ImmunoCAP and ISAC microarray in control individuals (CO), birch pollen-allergic patients with an oral allergy syndrome to apple (OAS⁺) and without apple allergy (OAS⁻). The recombinant form of the major apple allergen Mal d 1 discriminates between OAS patients and healthy control individuals. However, it has no advantage over conventional ImmunoCAP to rMal d 1 and does not discriminate between sensitization (OAS⁻) and real allergy to apple (OAS⁺).

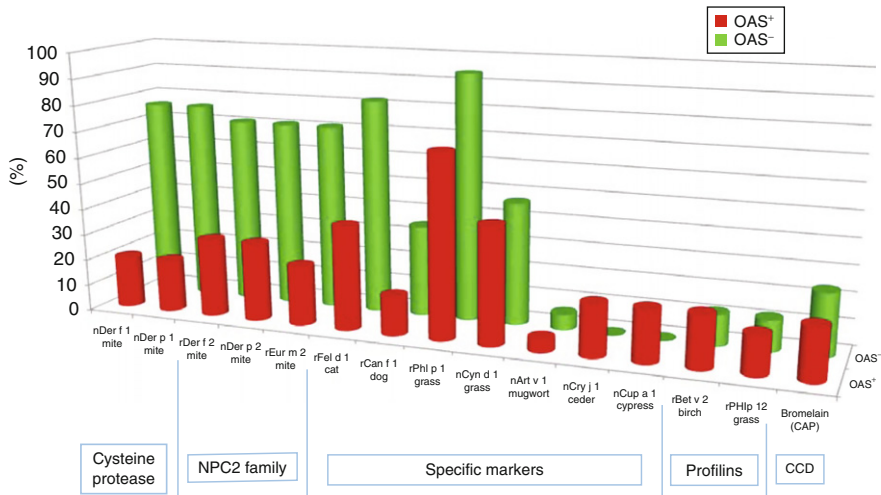


FIG. 3. Sensitization profiles to genuine markers of classical inhalant allergens in birch pollen-allergic patients with OAS to apple (OAS⁺) and without apple-related OAS (OAS⁻). Birch pollen-allergic patients without OAS to apple display a broader sensitization profile to classical inhalant allergens than birch pollen-allergic patients with an apple-related OAS. Abbreviations: Der f, *Dermatophagoides farinae*; Der p, *Dermatophagoides pteronyssinus*; Eur m, *Euroglyphus maynei*; Fel d, *Felis domesticus*; Can f, *Canis familiaris*; Phl p, *Phleum pratense*; Cyn d, *Cynodon dactylon*; Art v, *Artemisia vulgaris*; Cry j, *Cryptomeria japonica*; Cup a, *Cupressus arizonica*; Bet v, *Betula verrucosa*.

(*Hevea brasiliensis*). Some patients also displayed reactivity to the rubber particle-associated allergens Hev b 1 and/or Hev b 3. In contrast, none of the individuals without overt latex allergy or control individuals demonstrated IgE reactivity to rHev b 1, rHev b 3, rHev b 5, and rHev b 6.02 (Fig. 4). We demonstrated that three-quarters of the patients with clinically irrelevant latex sIgE antibodies displayed a positive microarray result for the recombinant latex profilin, rHev b 8 (Fig. 5). In contrast to the traditional ImmunoCAP bromelain assay, almost no sensitization to cross-reactive carbohydrates was demonstrated by the microarray. These observations greatly mirror the recent findings of Raulf-Heimsoth *et al.* [25].

In a third model (submitted data), the sensitization profile to hazelnut (*Corylus avellana*) allergens Cor a 1, Cor a 8, and Cor a 9 was investigated in infants with atopic dermatitis (AD) and at least one other food allergy of whom some showed sensitization to hazelnut. Of these hazelnut sensitized children, 60% demonstrated isolated IgE reactivity to the 11S legumin-like seed-storage protein from hazelnut, Cor a 9. None of the AD children

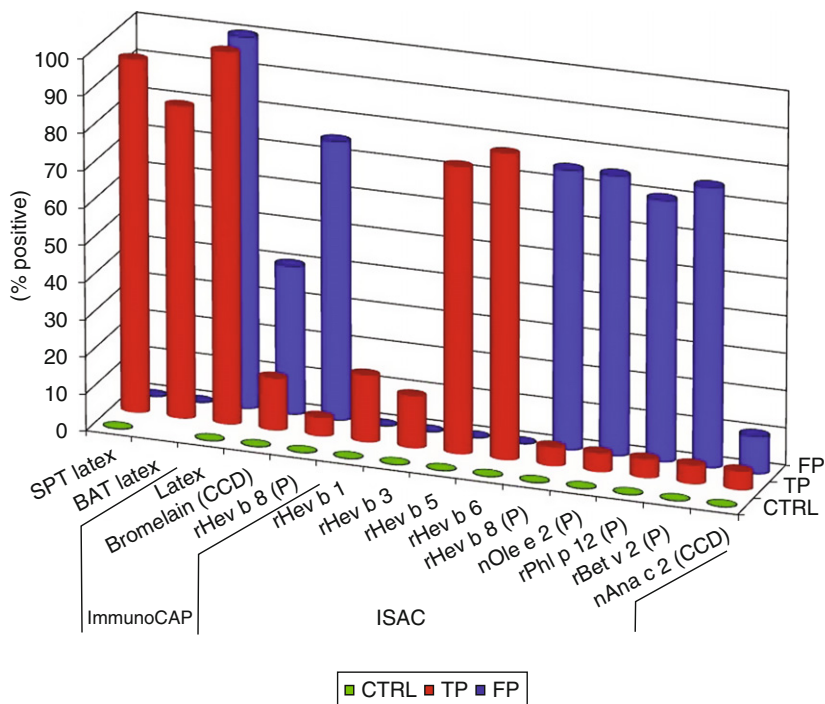


FIG. 4. Percentages of positive skin tests (SPT) and sIgE results by ISAC microarray and singleplexed ImmunoCAP to latex in control individuals (CTRL), latex-allergic patients (TP), and individuals sensitized to NRL (false positive, FP). Percentages of positive basophil activation tests (BAT) in TP and FP. Latex allergens: rubber elongation factor (Hev b 1), small rubber particle-associated protein (Hev b 3), acidic latex protein (Hev b 5), and hevein (Hev b 6). Profilins (P): latex (Hev b 8), olive (Ole e 2), timothy grass (Phl p 12), and birch (Bet v 2). Recombinant proteins are designated by “r” and native proteins by “n.” nAna c 2 is bromelain purified from pineapple (*Ananas comosus*) and is used as an indicator for sensitization to cross-reactive carbohydrate determinants (CCD). In all patients diagnosis of latex allergy could be documented by microarray CRD. Over three-quarters of the latex-allergic patients are sensitized to Hev b 5 and/or Hev b 6.02. Some patients also display reactivity to the rubber particle-associated allergens Hev b 1 and/or Hev b 3. None of the individuals without overt latex allergy or control individuals demonstrate IgE reactivity to rHev b 1, rHev b 3, rHev b 5, and rHev b 6.02. Three-quarters of the patients with clinically irrelevant latex sIgE antibodies display a positive microarray result to the recombinant latex profilin rHev b 8. In contrast to the ImmunoCAP bromelain assay, almost no sensitization to cross-reactive carbohydrates was demonstrated by the microarray.

without hazelnut sensitization showed positivity to Cor a 9. In previous studies sensitization to Cor a 9 was found in adult, Spanish hazelnut-allergic patients [30], or in children with systemic reactions [47].

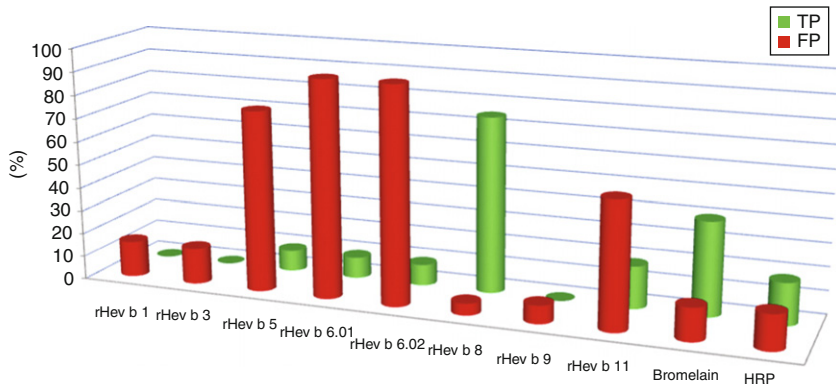


FIG. 5. Percentages of positive sIgE results for genuine and cross-reactive latex components, bromelain, and horseradish peroxidase (HRP) by singleplexed ImmunoCAP in patients (TP) and individuals sensitized to NRL (FP). Three-quarters of the patients with clinically irrelevant latex sIgE antibodies display a positive microarray result to recombinant latex profilin rHev b 8.

7. Perspectives

In analogy to the recent developments in CRD, IgE epitope mapping of allergens may be useful in providing information regarding a patient's sensitization profile and contribute to allergy diagnosis and prognosis. Recent studies have suggested a potential role for IgE-binding epitopes as biomarkers for characterizing various allergy phenotypes. Several studies regarding allergens in milk [48–50], wheat [51], egg [52], and peanut [53–55] have shown a correlation between IgE epitope diversity and severity or persistence of allergy. Several epitopes in milk allergens have been identified as candidate markers to predict the development of tolerance to milk [50]. IgE epitope mapping has the potential of becoming a new and additional tool for the diagnosis and prognosis of food allergy. However, further validation of current IgE-binding epitope identification methods is necessary before its application in the diagnosis of allergy is possible.

Recently, the discovery of the basophil activation marker CD63 induced the development of a flow cytometric technique to analyze allergen-specific *in vitro* activation of basophils (basophil activation test) [56]. The technique has proven to be a reliable instrument in the diagnosis of allergies to traditional inhalant allergens, natural rubber latex, hymenoptera venom [8, 57–60], and in the diagnosis of immediate drug allergy (for review, see Ref. [61]). Recently a few papers have been published in which the BAT is activated using purified or recombinant components such as the recombinant

lipid transfer wheat protein (rTri a 14) which can be used for the diagnosis of baker's asthma [62]. Gamboa and coworkers [33] compared the conventional peach extract with natural and recombinant allergens in the diagnosis of peach allergy, revealing a high specificity and sensitivity (72%) using rPru p 3. In the future, in analogy to CRD, the BAT as functional test may be utilized 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. However, further research and validation of this technique will be necessary.

8. Conclusion

Although CRD by microarray format seems promising and can provide the physician with a reliable tool for allergy diagnosis, its diagnostic performance requires further assessment and validation using well-defined clinical samples. At present important allergenic sources such as wasp venom, walnut, and almond are missing. It is anticipated that additional and alternative components will further optimize the performance of the assay. As long as not all relevant allergen molecules are available in sufficient number and quality, a combination of microarray and singleplexed sIgE will be necessary for allergy diagnosis. On the other hand, the physician can acquire information from a single run that largely exceeds the output from conventional singleplexed IgE capturing tools. At this point, the major challenge will be to accurately translate the variety of quantitative sIgE results, determine their clinical relevance, and identify particular sensitization patterns that might lead to diagnostic algorithms.

Up to now, only a limited number of studies have evaluated the application of the microarray in clinical practice using well-defined patient groups. Therefore, adequately powered and comprehensive clinical trials, in well-defined patient groups including sensitized individuals without clinical allergy, are required before the technique can enter mainstream clinical application.

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REFERENCES

- [1] R.G. Hamilton, A.N. Franklin Jr., In vitro assays for the diagnosis of IgE-mediated disorders, *J. Allergy Clin. Immunol.* 114 (2004) 213–225.
- [2] L. Cox, B. Williams, S. Sicherer, et al., Pearls and pitfalls of allergy diagnostic testing: report from the American College of Allergy, Asthma and Immunology/American Academy of Allergy, Asthma and Immunology Specific IgE Test Task Force, *Ann. Allergy Asthma Immunol.* 101 (2008) 580–592.
- [3] C.H. Blackley, Hay Fever: Its Causes Treatment and Effective Prevention, Ballière, London, 1880.
- [4] K. Ishizaka, T. Ishizaka, M.M. Hornbrook, Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity, *J. Immunol.* 97 (1966) 75–85.
- [5] L. Wide, H. Bennich, S.G. Johansson, Diagnosis of allergy by an in-vitro test for allergen antibodies, *Lancet* 2 (1967) 1105–1107.
- [6] M. Wensing, J.H. Akkerdaas, W.A. van Leeuwen, et al., IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance, *J. Allergy Clin. Immunol.* 110 (2002) 435–442.
- [7] A. Mari, IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity, *Int. Arch. Allergy Immunol.* 129 (2002) 286–295.
- [8] D.G. Ebo, M.M. Hagendorens, C.H. Bridts, L.S. De Clerck, W.J. Stevens, Sensitization to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of allergy, *Clin. Exp. Allergy* 34 (2004) 137–144.
- [9] R. van Ree, Clinical importance of cross-reactivity in food allergy, *Curr. Opin. Allergy Clin. Immunol.* 4 (2004) 235–240.
- [10] V. Mahler, C. Gutgesell, R. Valenta, T. Fuchs, Natural rubber latex and hymenoptera venoms share ImmunoglobulinE-epitopes accounting for cross-reactive carbohydrate determinants, *Clin. Exp. Allergy* 36 (2006) 1446–1456.
- [11] F. Altmann, The role of protein glycosylation in allergy, *Int. Arch. Allergy Immunol.* 142 (2007) 99–115.
- [12] H. Malandain, F. Giroux, Y. Cano, The influence of carbohydrate structures present in common allergen sources on specific IgE results, *Eur. Ann. Allergy Clin. Immunol.* 39 (2007) 216–220.
- [13] W.R. Thomas, G.A. Stewart, R.J. Simpson, et al., Cloning and expression of DNA coding for the major house dust mite allergen Der p 1 in *Escherichia coli*, *Int. Arch. Allergy Appl. Immunol.* 85 (1988) 127–129.
- [14] R. Valenta, J. Lidholm, V. Niederberger, B. Hayek, D. Kraft, H. Gronlund, The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT), *Clin. Exp. Allergy* 29 (1999) 896–904.
- [15] L. Kazemi-Shirazi, V. Niederberger, B. Linhart, J. Lidholm, D. Kraft, R. Valenta, Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy, *Int. Arch. Allergy Immunol.* 127 (2002) 259–268.
- [16] R. Hiller, S. Laffer, C. Harwanegg, et al., Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment, *FASEB J.* 16 (2002) 414–416.
- [17] J. Lidholm, B.K. Ballmer-Weber, A. Mari, S. Vieths, Component-resolved diagnostics in food allergy, *Curr. Opin. Allergy Clin. Immunol.* 6 (2006) 234–240.
- [18] N. Mothes, R. Valenta, S. Spitzauer, Allergy testing: the role of recombinant allergens, *Clin. Chem. Lab. Med.* 44 (2006) 125–132.

- [19] S. Steckelbroeck, B.K. Ballmer-Weber, S. Vieths, Potential, pitfalls, and prospects of food allergy diagnostics with recombinant allergens or synthetic sequential epitopes, *J. Allergy Clin. Immunol.* 121 (2008) 1323–1330.
- [20] S. Heiss, V. Mahler, R. Steiner, et al., Component-resolved diagnosis (CRD) of type I allergy with recombinant grass and tree pollen allergens by skin testing, *J. Invest. Dermatol.* 113 (1999) 830–837.
- [21] R.E. Rossi, G. Monasterolo, S. Monasterolo, Measurement of IgE antibodies against purified grass-pollen allergens (Phl p 1, 2, 3, 4, 5, 6, 7, 11, and 12) in sera of patients allergic to grass pollen, *Allergy* 56 (2001) 1180–1185.
- [22] R. Moverare, K. Westritschnig, M. Svensson, et al., Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization, *Int. Arch. Allergy Immunol.* 128 (2002) 325–335.
- [23] G. Pittner, S. Vrtala, W.R. Thomas, et al., Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens, *Clin. Exp. Allergy* 34 (2004) 597–603.
- [24] B.K. Ballmer-Weber, A. Wangorsch, B. Bohle, et al., Component-resolved in vitro diagnosis in carrot allergy: does the use of recombinant carrot allergens improve the reliability of the diagnostic procedure? *Clin. Exp. Allergy* 35 (2005) 970–978.
- [25] M. Raulf-Heimsoth, H.P. Rihs, P. Rozynek, et al., Quantitative analysis of immunoglobulin E reactivity profiles in patients allergic or sensitized to natural rubber latex (*Hevea brasiliensis*), *Clin. Exp. Allergy* 37 (2007) 1657–1667.
- [26] J.D. Ramos, G.R. Valmonte, R.M. de Guia, Recombinant proteins and peptides as diagnostic and therapeutic reagents for arthropod allergies, *Protein Pept. Lett.* 14 (2007) 992–1002.
- [27] D. Barber, F. de la Torre, F. Feo, et al., Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study, *Allergy* 63 (2008) 1550–1558.
- [28] R. Asero, L. Jimeno, D. Barber, Component-resolved diagnosis of plant food allergy by SPT, *Eur. Ann. Allergy Clin. Immunol.* 40 (2008) 115–121.
- [29] C. Constantin, S. Quirce, M. Poorafshar, et al., Micro-arrayed wheat seed and grass pollen allergens for component-resolved diagnosis, *Allergy* 64 (2009) 1030–1037.
- [30] K.S. Hansen, B.K. Ballmer-Weber, J. Sastre, et al., Component-resolved in vitro diagnosis of hazelnut allergy in Europe, *J. Allergy Clin. Immunol.* 123 (2009) 1134–1141.
- [31] M. Fernandez-Rivas, C. Benito, E. Gonzalez-Mancebo, D.A. de Durana, Allergies to fruits and vegetables, *Pediatr. Allergy Immunol.* 19 (2008) 675–681.
- [32] A.E. Flinterman, J.H. Akkerdaas, A.C. Knulst, R. van Ree, S.G. Pasmans, Hazelnut allergy: from pollen-associated mild allergy to severe anaphylactic reactions, *Curr. Opin. Allergy Clin. Immunol.* 8 (2008) 261–265.
- [33] P.M. Gamboa, M.L. Sanz, M. Lombardero, et al., Component-resolved in vitro diagnosis in peach-allergic patients, *J. Investig. Allergol. Clin. Immunol.* 19 (2009) 13–20.
- [34] R. Valenta, T. Twaroch, I. Swoboda, Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area, *J. Investig. Allergol. Clin. Immunol.* 17 (Suppl. 1) (2007) 36–40.
- [35] K. Deinhofer, H. Sevcik, N. Balic, et al., Microarrayed allergens for IgE profiling, *Methods* 32 (2004) 249–254.
- [36] C. Harwanegg, R. Hiller, Protein microarrays for the diagnosis of allergic diseases: state-of-the-art and future development, *Clin. Chem. Lab. Med.* 43 (2005) 1321–1326.
- [37] D.G. Ebo, C.H. Bridts, M.M. Verweij, et al., Sensitization profiles in birch pollen-allergic patients with and without oral allergy syndrome to apple: lessons from multiplexed component-resolved allergy diagnosis, *Clin. Exp. Allergy* 40 (2010) 339–347.

- [38] D.G. Ebo, M.M. Hagendorens, K.J. De Knop, et al., Component resolved diagnosis from latex allergy by microarray, *Clin. Exp. Allergy* 40 (2010) 348–358.
- [39] B. Jahn-Schmid, C. Harwanegg, R. Hiller, et al., Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E, *Clin. Exp. Allergy* 33 (2003) 1443–1449.
- [40] S. Wöhrl, K. Vigl, S. Zehetmayer, et al., The performance of a component-based allergen-microarray in clinical practice, *Allergy* 61 (2006) 633–639.
- [41] R. Leonard, B.O. Petersen, M. Himly, et al., Two novel types of *O*-glycans on the mugwort pollen allergen Art v 1 and their role in antibody binding, *J. Biol. Chem.* 280 (2005) 7932–7940.
- [42] H. Ott, J.M. Baron, R. Heise, et al., Clinical usefulness of microarray-based IgE detection in children with suspected food allergy, *Allergy* 63 (2008) 1521–1528.
- [43] G. Gadermaier, N. Wopfner, M. Wallner, et al., Array-based profiling of ragweed and mugwort pollen allergens, *Allergy* 63 (2008) 1543–1549.
- [44] A. Yagami, Y. Inaba, Y. Kuno, et al., Two cases of pollen-food allergy syndrome to soy milk diagnosed by skin prick test, specific serum immunoglobulin E and microarray analysis, *J. Dermatol.* 36 (2009) 50–55.
- [45] A. Adachi, T. Horikawa, H. Shimizu, et al., Soybean beta-conglycinin as the main allergen in a patient with food-dependent exercise-induced anaphylaxis by tofu: food processing alters pepsin resistance, *Clin. Exp. Allergy* 39 (2009) 167–173.
- [46] D.G. Ebo, M.M. Hagendorens, C.H. Bridts, A.J. Schuerwegh, L.S. De Clerck, W.J. Stevens, Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy, *Cytometry B Clin. Cytom.* 64 (2005) 28–33.
- [47] K. Beyer, G. Grishina, L. Bardina, A. Grishin, H.A. Sampson, Identification of an 11S globulin as a major hazelnut food allergen in hazelnut-induced systemic reactions, *J. Allergy Clin. Immunol.* 110 (2002) 517–523.
- [48] J. Lin, L. Bardina, W.G. Shreffler, et al., Development of a novel peptide microarray for large-scale epitope mapping of food allergens, *J. Allergy Clin. Immunol.* 124 (2009) 315–322.
- [49] K.M. Jarvinen, K. Beyer, L. Vila, P. Chatchatee, P.J. Busse, H.A. Sampson, B-cell epitopes as a screening instrument for persistent cow's milk allergy, *J. Allergy Clin. Immunol.* 110 (2002) 293–297.
- [50] K. Beyer, K.M. Jarvinen, L. Bardina, et al., IgE-binding peptides coupled to a commercial matrix as a diagnostic instrument for persistent cow's milk allergy, *J. Allergy Clin. Immunol.* 116 (2005) 704–705.
- [51] H. Matsuo, K. Kohno, H. Niihara, E. Morita, Specific IgE determination to epitope peptides of omega-5 gliadin and high molecular weight glutenin subunit is a useful tool for diagnosis of wheat-dependent exercise-induced anaphylaxis, *J. Immunol.* 175 (2005) 8116–8122.
- [52] K.M. Jarvinen, K. Beyer, L. Vila, L. Bardina, M. Mishoe, H.A. Sampson, Specificity of IgE antibodies to sequential epitopes of hen's egg ovomucoid as a marker for persistence of egg allergy, *Allergy* 62 (2007) 758–765.
- [53] W.G. Shreffler, K. Beyer, T.H. Chu, A.W. Burks, H.A. Sampson, Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes, *J. Allergy Clin. Immunol.* 113 (2004) 776–782.
- [54] W.G. Shreffler, D.A. Lencer, L. Bardina, H.A. Sampson, IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2, *J. Allergy Clin. Immunol.* 116 (2005) 893–899.

- [55] A.E. Flinterman, E.F. Knol, D.A. Lencer, et al., Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy, *J. Allergy Clin. Immunol.* 121 (2008) 737–743.
- [56] E.F. Knol, F.P. Mul, H. Jansen, J. Calafat, D. Roos, Monitoring human basophil activation via CD63 monoclonal antibody 435, *J. Allergy Clin. Immunol.* 88 (1991) 328–338.
- [57] D.G. Ebo, J. Sainte-Laudy, C.H. Bridts, et al., Flow-assisted allergy diagnosis: current applications and future perspectives, *Allergy* 61 (2006) 1028–1039.
- [58] D.G. Ebo, C.H. Bridts, M.M. Hagendorens, N.E. Aerts, L.S. De Clerck, W.J. Stevens, Basophil activation test by flow cytometry: present and future applications in allergology, *Cytometry B Clin. Cytom.* 74B (2008) 201–210.
- [59] A.L. De Weck, M.L. Sanz, P.M. Gamboa, et al., Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls, *Int. Arch. Allergy Immunol.* 146 (2008) 177–189.
- [60] A.L. De Weck, M.L. Sanz, P.M. Gamboa, et al., Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. II. Technical issues, *J. Investig. Allergol. Clin. Immunol.* 18 (2008) 143–155.
- [61] D.G. Ebo, M.M. Hagendorens, C.H. Bridts, L.S. De Clerck, W.J. Stevens, The basophil activation test in immediate drug allergy, *Acta Clin. Belg.* 64 (2009) 129–135.
- [62] A. Palacin, J. Varela, S. Quirce, et al., Recombinant lipid transfer protein Tri a 14: a novel heat and proteolytic resistant tool for the diagnosis of baker's asthma, *Clin. Exp. Allergy* 39 (2009) 1267–1276.