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REVIEW

Microarrays: Molecular allergology and nanotechnology for personalised medicine (II)

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Abstract
Progress in nanotechnology and DNA recombination techniques have produced tools for the diagnosis and investigation of allergy at molecular level. The most advanced examples of such progress are the microarray techniques, which have been expanded not only in research in the field of proteomics but also in application to the clinical setting.
Microarrays of allergic components offer results relating to hundreds of allergenic components in a single test, and using a small amount of serum which can be obtained from capillary blood. The availability of new molecules will allow the development of panels including new allergenic components and sources, which will require evaluation for clinical use.
Their application opens the door to component-based diagnosis, to the holistic perception of sensitisation as represented by molecular allergy, and to patient-centred medical practice by allowing great diagnostic accuracy and the definition of individualised immunotherapy for each patient.
The present article reviews the application of allergenic component microarrays to allergology for diagnosis, management in the form of specific immunotherapy, and epidemiological studies. A review is also made of the use of protein and gene microarray techniques in basic research and in allergological diseases. Lastly, an evaluation is made of the challenges we face in introducing such techniques to clinical practice, and of the future perspectives of this new technology.

The clinical use of allergenic component microarrays

Microarrays of allergic components offer results relating to hundreds of allergenic components in a single test, and using a small amount of serum which can be obtained from capillary blood. By increasing the level of analytical detail, allergenic component-resolved diagnosis (CRD) microarrays afford an image of patient sensitisation at molecular level – making it possible to distinguish between major and minor (but equally important in terms of disease) allergens. In this context, by also identifying the panallergens, the true sensitisation profile (mono-, oligo- or polysensitisation) can be established.

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This broad, holistic view will make it possible to monitor the IgE reactivity profiles during the development and course of the disease and its treatment, evaluating the correlation between the changes in these profiles and the patient clinical parameters (e.g., progression of allergic rhinoconjunctivitis to asthma). Thanks to the use of molecular allergenic markers, such applications may help predict the risk of developing new allergic disorders or their intensity (e.g., increased risk of anaphylaxis due to rosacea associated to LTP,[1] of asthma associated to Ole e 2 and Ole e 10,[2] or a lesser risk in people allergic to latex if they are only allergic to Hev b 8),[3] and establish the origin of sensitisation (e.g., latex, according to the pattern of its allergenic components).[4,5]

This knowledge of the molecular sensitisation profile can already be used to individualise the composition of extracts for specific immunotherapy with great accuracy, by discovering cross-sensitisations to panallergens which lack clinical relevance. Likewise, allergenic microarrays could be used in the context of a ''pharmacoproteomic'' approach to monitor the efficacy of specific immunotherapy, assessing the evolution of allergen-specific IgE/IgG or the appearance of new sensitisations during treatment which could have an impact upon the clinical course.

Diagnosis

Food allergy

Many studies indicate that the molecular analysis of allergenic sensitisation patterns can be of increased clinical usefulness in diagnosing IgE-mediated allergic processes. Purified natural as well as recombinant allergens have been used to this effect, together with synthetic peptide panels.[6]

Different studies have shown microarrays to be useful in allergic patients for identifying cow's milk and egg allergen patterns[7,8]. In this sense it is essential for the technique to be applicable to the management of patients with food allergy, with the prediction of when a given potentially allergic patient will develop a reaction after ingestion of the culprit food allergen.

These clinical decision points (CDPs) offering certain positive predictive values (PPVs) in application to oral provocation are regarded as important parameters for diagnosing paediatric food allergy,[9–11] and the combined microarray findings for egg and cow's milk in different age groups have been able to yield PPVs for 90%, 95% and 99% of the patients.[8]

Beyer et al. determined disease persistence by screening children with allergy to cow's milk using a microarray casein peptide panel.[12]

The technique has also been shown to be useful in application to peanut allergy,[13] where a correlation is observed between the diversity of specific IgE and the severity of the allergic clinical manifestations, as well as the different epitopes of these allergenic molecules.[14] The microarray technique has been useful for studying the allergenic potential of two 2 S albumins from soybean.[15]

Incorporation is required of new allergens such as Ara h 9, which in the peanut allergy population constitutes a minor allergen, but is more important in patients from south-eastern Europe, where sensitisation to the classical major allergens – Ara h 1, Ara h 2, and Ara h 3 – is less pronounced.[16]

The microarray technique as a screening test has served to classify the phenotype of pollen-food allergy syndrome in patients with oral allergy to soy.[17]

Allergy to aeroallergens

Deinhofer et al.[18] demonstrated that for a well characterised and selected panel of recombinant pollen allergens, the microchip-based diagnosis of allergy can equal the performance of the well-established tests in terms of sensitivity and specificity – with excellent correlations for recombinant birch and grass allergens. Wöhrl et al.[19] in turn confirmed the diagnostic usefulness of a microarray involving a broader range of inhaled allergen components.

The pollens of Ambrosia and Artemisia share allergens – profilin, polcalcin and nonspecific lipid transfer protein (nsLTP) – which cause cross-reactivity between them.[20] In a study of 509 patients, 93% of the individuals sensitised to Artemisia were also found to be sensitised to Ambrosia, while 48% of those sensitised to Ambrosia were also sensitised to Artemisia.[21] In patients of this kind, the study of the allergenic components of both types of pollen based on microarray tests has been shown to be as useful as ELISA testing – yielding information on the differential diagnosis between one type of sensitisation and the other, of great importance for selecting the most appropriate allergenic source for immunotherapy.[22]

This technique has been used to study the sensitisation profiles pollen-vegetable food syndromes such as birch-apple syndrome,[23] or to establish the differential diagnosis of a type of occupational asthma (baker's asthma).[24]

Microarrays have also been found to be useful in differentiating primary sensitisations to pollen, fruits and latex from the subclinical immune recognition of different prophyllins and LTP in paediatric patients.[25]

Allergy to insect venom

Protein microarrays can also be used for the study of allergy to bee, wasp and ant venoms.[26]

Immunotherapy

In allergen-specific immunotherapy, identification of the allergens causing the disease is a fundamental requirement for precise treatment prescription. This is particularly important when considering that over 60% of all pollen allergic patients are polysensitised. In this setting, distinguishing panallergens (profilins, polcalcins) and homologous molecules of limited presence (e.g., LTP) from highly selective components results in improved immunotherapy.[27]

A number of studies have shown that recombinant allergens can be used to distinguish patients who can benefit from immunotherapy (monosensitised patients) from those showing cross-reactions with an allergen from a different biological source.[28,29] Furthermore, purified

allergenic markers can be used to monitor the immune effects of treatment and prevent new sensitisations during immunotherapy.[30,31]

In all these aspects, allergenic component microarrays are of great importance. In addition, the fact that the molecular sensitisation profile of the patient can be known makes it logical to define the next step as the introduction of ''molecular'' immunotherapy adapted to the specific sensitisation profile of each individual patient.[32–34]

In this sense, clinical trials have been made using recombinant allergens as either the sum of recombinants that conform a natural extract,[35] or as recombinant allergens modified to exhibit reduced allergenic activity [36–38]– showing also that immunotherapy targeted to recombinant allergens can be both effective and safe. Recently, a new specific immunotherapeutic treatment targeted to Alternaria with purified native allergen (nAlt a 1) has been introduced on the market, showing good immunogenicity and safety.[39]

In this context it is necessary to demonstrate that such formulations possess the same potency as the complete natural allergens, and that the panel of recombinant allergens encompasses all the epitopes recognised by the B and T cells. In the case of gramineous species (grasses), it has been shown that a panel of 5 recombinants (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b), or the mentioned panel plus Phl p 6, significantly reduces the symptoms and induces a specific and very intense response against these allergens.[35] Birch allergens behave in a similar way.

It has been shown that recombinant hybrids from unrelated allergenic sources possess great immunogenic capacity.[40]

In patients sensitised to dust mites, the technique already allows us to determine whether they are sensitised to group 1 or to group 2 allergens, or whether in contrast the subjects are sensitised to tropomyosin – which would constitute a contraindication to immunotherapy with dust mite extracts containing group 1 and 2 allergens.[26]

Epidemiological studies

''Universal'' allergenic microarrays may offer a means for determining specific IgE reactivity in concrete regions or populations, specific disease patterns, or the dynamic evolution of sensitisations – contributing much more detailed information.[41]

Given the increase in the prevalence of allergic disease in children and the possibility of sensitisation during the foetal period, studies have been made of the relationships between genes, and between these and the environment, based on the use of arrays. The findings suggest that the control of maternal atopy and the prenatal redox response may prevent IgE-mediated diseases.[42]

It has been shown that early sensitisation to a common allergen (e.g., egg, grass pollen, dust mites or cat dander) is associated with an increased risk of developing asthma.[43] The Section on Pediatrics of the European Academy of Allergy and Clinical Immunology (EAACI) has established the criteria for allergy detection studies in children, with a view to defining a strategy which can help prevent the course of allergy.[44]

Since part of the primary preventive measures have been shown to be scantly effective,[45] new approaches and lines of research are needed[46] to understand which factors influence the development of these diseases. In this context, allergen microarrays, thanks to the extremely low volume of sample required (20 μl) and the large number of allergens which can be studied, offer a tool for improved risk evaluation in atopic individuals in the early stages of life.

Use of gene and protein microarrays in allergology

Microarray technology has become the most potent instrument for analysing genic expression profiles and functional genomics. In this context, a range of basic and applied research lines have been developed in the field of allergic diseases, in which microarray testing is a critical tool.

Basic research

Microarray technology has been used for the study of interactions among allergenic proteins, immunoglobulins and T cell receptors, with a view to developing genetic modifications which can yield hypoallergenic variants of plant proteins.[47]

Microarrays have been applied in the investigation of clonal diversity[48] and immune response heterogeneity among patients,[49] and to establish the clinical correlations between antibody diversity and the allergic manifestations.[50–52]

Microarray technology has been shown to be very useful for mapping and characterising allergenic epitopes.[53] An example is represented by cow's milk allergens,[54] with differentiation between IgE and IgG4 patterns for sequential epitopes of alpha(s1)-, alpha(s2)-, beta- and kappa-caseins and beta-lactoglobulin in reactive patients and tolerant individuals.[55] However, further studies are needed to assess the potential usefulness of the technique in clinical practice.

In immunology, microarrays offer applications in the extensive field of genic expression in primary immune deficiencies.[56]

Atopic dermatitis

Noh et al.[57] in studying the relationship between atopic dermatitis and food allergy, measured the concentration of IgE and IgG 4 specific of certain foods, using an allergenic protein chip (Allergy Chip). The authors suggested that this protein microarray technique could be one of the most useful methods for evaluating allergic condition in such diseases.

Its usefulness in diagnosing sensitisations in patients with atopic dermatitis has also been recently demonstrated by Ott et al.[58]

Likewise, antibody microarrays have been used to study the cytokines and chemokines implicated in the physiopathology of atopic dermatitis in children,[59] with the use of biochips for the molecular genomic analysis of this disease.[60]

Contact dermatitis

Large-scale genetic expression analysis based on the use of DNA microarrays has been used to study the genes implicated in contact dermatitis. The detected genetic markers can be posteriorly included in microarrays on a smaller scale, to establish the definitive diagnostic test.[61]

Rhinitis

Microarrays have helped to determine genic expression in allergic rhinitis,[62] to study the pathogenesis of the disease, and to evaluate the role of chemokines and their receptors via the expression of their complementary DNA (cDNA) in the nasal mucosa, using gene biochips.[63]

Asthma

In asthma, microarray technology has been used to study the pathogenesis of the disease, yielding new biological diagnostic markers and therapeutic targets, and furthering our understanding of the pathogenic mechanisms of bronchial asthma.[64,65] In application to allergic asthma complex, microarrays have been shown to be valid and relevant in defining allergic asthma expression patterns, comparing two different types of biochips (U95Av2 and U133A Gene Chips) in the study of bronchial biopsy samples.[66]

Microarrays have been used to explore the importance of interleukin 13 (IL-13) in the pathogenesis of asthma.[67] Another study, involving cytokine microarrays, has shown cytokine PARC/CCL18 to be a new factor implicated in the development of eosinophilic inflammation of the airways in asthma.[68]

Asthma exacerbations requiring hospital admission in children have been associated to viral infections occurring mainly in atopic individuals, although the significance of such comorbidities is not known. A study using flow cytometry and genic expression microarrays has suggested that viral infection in atopic children may trigger an atopic inflammatory cascade which amplifies and maintains the airway inflammation initiated by innate antiviral immunity via use of the underlying atopic mechanisms. These interactions may explain the susceptibility of atopic patients to severe asthma episodes induced by viral infections.[69]

Likewise, microarrays offer great potential for studying the pharmacogenomics of asthma in the genetic polymorphisms which influence the results of asthma therapy.[70] Recent studies have revealed the genes associated to asthma and to a favourable response to inhaled corticosteroids in bronchial epithelial cells[71] and in peripheral blood mononuclear cells.[72] Likewise, if pharmacogenetic studies were able to establish the genetic differences in patients with a good response to fluticasone or montelukast,[73,74] it could become possible to avoid the excessive or insufficient treatment of such individuals – microarray technology in turn facilitating the individualisation of therapy.

T cell microarrays are used to assess the activity of the genes mediating apoptosis in the context of tolerance – this being useful for evaluating the efficacy of immunotherapy, particularly as regards the sublingual route in peanut allergy.[75]

Challenges for clinical application

In order for microarray testing to become a routine technique in allergy, its performance and diagnostic accuracy must be confirmed by large-scale, population-based multicentre studies reflecting the prevalence of the disease and the setting in which the mentioned technology will be posteriorly used.

In a time of increasing control of healthcare expenditure, new technologies must be evaluated to confirm that they offer the expected added value and cost/benefit ratio. This technology uses allergens which must be purified or created with recombinant technology, and moreover requires sophisticated chip analytical instruments. However, with use, the cost of this technology will tend to decrease. On the other hand, the cost must be weighed against the great amount of information obtained, the small sample volume required, and particularly the probable benefit of determining the prognosis and contributing to prevention and the effectiveness and safety of treatment, by allowing selection of the most appropriate allergen composition for immunotherapy. Cost/utility studies are therefore needed, with the comparison of effectiveness versus the rest of the in vitro and in vivo diagnostic techniques currently available.[76]

In turn, it must be checked that transfer of the technology from the research setting to clinical practice (and from evidence to clinical application) proves satisfactory [77]. For example: despite their amplitude, the existing allergen panels do not contain all the components of clinical interest of the respective allergen sources, or all the sources that are of clinical relevance. Additional clinical studies are needed to improve allergen panel definition.

Likewise, clinical decision support is needed, since the large volume of information obtained is complex to interpret. In this sense, the microarray test results should offer help for clinicians in the form of specific software, with direct access to expert databases and guides, making it possible to better interpret the tests and their application to therapy.

Conclusions and future perspectives

Allergenic component microarrays offer an elegant way to avoid the problem of allergen standardisation and false polysensitisation.

However, this novel technology has further implications: it represents a true change in paradigm, offering a different view of the aetiology of allergic disease and extending the approach from allergenic sources to allergenic molecules – from botanical classification to molecular classification.[78] This systematisation in turn should prove manageable, since it involves a limited number of protein families and functions,[79,80] thus allowing a holistic approach to the disease.

This technology opens the door to personalised medical practice, by allowing diagnosis and planning at molecular level, specific for each patient, with a known and balanced dosing of standardised allergens for immunotherapy. In this context, it is possible to include allergens that are not well represented in the natural extracts but which are

nevertheless of clinical relevance. Alternatively, genetic engineering can be used to incorporate hypoallergenic variants with lesser IgE reactivity, but which result in equal or even superior T cell reactivity – thus separating immunogenicity from allergenicity, and making the technology both more effective and safer.

This novel diagnostic technology is minimally invasive, makes use of small sample volumes, offers quantitative results, and constitutes a multianalytic test – thus facilitating its incorporation to clinical use. However, not all patients will require such testing, since in clinical practice studies at molecular level are mainly recommended in individuals sensitised to panallergens detected by prick testing,[81] or in polysensitised patients in which diagnostic doubts persist.

In an even broader sense, the use of microarrays for exploring these complex disease processes will reveal the aetiopathogenic implication of multiple genes, helping to clarify the many and varied mechanisms and associated pathological pathways – the complexity of which requires the application of network theory[82] to allow understanding. This has given rise to a new field in biology – the biology of systems – which aims to decipher the cell and signal networks underlying these biological processes, using gene and protein microarrays as its principal tool.

Application of the new knowledge afforded by nanotechnology on an individualised basis in allergic patients requires specialised knowledge that does not form part of the standard curriculum of clinical allergologists at the present time. In this context, a training effort in both the clinical and research settings is warranted, with a view to developing this new era in allergology.

Conflict of interest

The author declares no conflicts of interest.

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