

## PROTEIN MICROARRAYS FOR DIAGNOSIS AND RESEARCH OF ALLERGIC DISEASES

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*Summary:* For both the systematic as well as for the system-wide analysis of protein function, interaction and abundance, protein microarrays represent one of the pillars underlying modern high-throughput Proteomics. The prospect of analysing samples in a highly parallel while miniaturized fashion has fuelled efforts to develop novel diagnostic applications in the cancer, autoimmune or allergy field. Here, I discuss recent advancements in the development of protein microarrays for the profiling of IgE antibodies in the diagnosis of Type 1-related allergic diseases.

*Key words:* allergy, biochip, component resolved diagnosis, IgE, microarray, molecular diagnostics, recombinant allergen

### Protein microarrays as promising tools in proteomics

Following the publication of the first draft of the human genome sequence in 2001, interest into the functional analysis of the information stored in our genetic make-up has grown tremendously. Efforts to investigate the interplay of gene-products on a system-wide scale have gained a lot of support by the technological advancements made available by DNA microarray technology which has become a widespread tool for large-scale RNK expression profiling (1) and genotyping analysis (2). To study the expression, function and interaction of the proteins stored in the entire genome (i.e., the proteome) is only the logical next step in providing a better understanding of the mechanisms of cellular function, disease development or drug action. Eventually, the term Proteome was first coined in 1996 (3) and Proteomics has consequently been called the study of the entire repertoire of proteins expressed in a given cell, tissue or body fluid.

Although high-throughput applications like DNA microarrays have been introduced as reliable tools for conducting research in the fields of genomics and transcriptomics, an increasing number of investigations indicate that the information about gene expression gained on the RNA level often correlates poorly with the abundance or function of the corresponding gene products (i.e., proteins) (4). Therefore, the need for a large-scale collection of protein expression data and for the understanding of the complex network of protein interaction and functioning on a cellular level has been boosting the demand for large-scale and high-throughput methods in the area of Proteomics tremendously. Next to 2D PAGE and mass spectrometry, the prospect of developing protein microarrays containing hundreds or even thousands of individual antibodies or proteins for the functional investigation of the proteome has raised considerable interest among scientists in academia and industry worldwide.

In comparison to the routine and throughput that is achievable in today's large-scale analysis of entire genomes, Proteomics still remains a multifaceted and rapidly evolving research area (5). Consequently, a number of different analytical approaches are being used today, such as two-dimensional gel electrophoresis (6), mass spectrometry (MS) (7), quantitative protein profiling (8), sensitive multi-dimensional protein identification technologies (9, 10), or MS-based protein marker profiling (11, 12).

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Protein microarrays, i.e. multiplex solid-phase immunoassays in a miniaturized form, represent just one of the emerging application in Proteomics (13, 14). In principle, protein biochips are the counterparts of DNA biochip technology, using spatially separated and individually addressable microspots of antibodies (15), proteins (16), small molecules (17) or cell extracts (18) contained in a microarray to monitor the function, interaction or expression of (protein) analytes of interest. Apart from the power of analyzing protein function on the proteome level, the prospect of developing diagnostic applications has become an attractive goal for researchers in the protein microarray field. For the development of novel diagnostic applications, protein microarray technology has been employed to immobilize purified (natural or recombinant) antigens or antibodies as capturing agents for the screening of analytes (e.g. IgEs or IgGs) in the serum of diseased patients. Examples include the monitoring of autoantibody responses in autoimmune diseases (19, 20), *alopecia areata* (21), diabetes (22), systemic lupus erythematosus (23), allergy (16, 24–26), cancer (27), rheumatoid arthritis (28), and the profiling of linear allergen epitopes (29, 30).

Like their DNA biochip counterparts, protein microarrays are usually built on planar substrates, such as high-quality glass microscopy slides, silicon wafers or plastic devices. For the stable and functional immobilisation of proteins, the surface of the substrate is usually modified in order to bind protein compounds in a stable and biologically active manner. The latter is achieved by adding chemical modifications to the surface, or by the application of 3D-like functional layers, such as nitrocellulose or hydrogels. Different types and numbers of capture molecules (e.g. native or recombinant proteins, antibodies, peptides or aptamer molecules) are subsequently microarrayed on these substrates by robotic equipment in order to create individually addressable reaction sites (spots, features). Each of these (usually)  $\mu\text{m}$ -sized spots is later employed in a miniaturised ligand binding assay where each biological interaction can subsequently be monitored by applying specific detection antibodies (e.g. labelled with a fluorescence dye) combined with sensitive detection methods (e.g. fluorescence laser scanning microscopes). As a result fluorescence images are produced, and the signal intensities of each spot can be used for analyte quantification, to determine protein expression levels, or to monitor the function of specific enzyme-substrate pairs (31). Although the concept of miniaturised ligand binding assays was described almost 15 years ago (32), current technological solutions for developing protein microarrays have mostly remained in a conceptual state. Among several reasons, this is due to the high level of complexity intrinsic to the antigens employed in miniaturised assays (e.g. size, charge, solubility, surface activity, 3D structure), the lack of efficient high-throughput recombinant protein expression platforms or the difficulty of raising antigen-specific binding agents against the epitope complexity found in entire proteomes.

## Type 1 allergic diseases

Type 1 IgE-related allergy represents a major health burden of industrialised nations (USA, Europe and Japan) where an estimated 25% of the population suffers from IgE-mediated atopic diseases (e.g. asthma, rhinitis, atopic dermatitis, conjunctivitis, or sinusitis). Type 1 allergy is initiated by the generation of IgE antibodies in response to the primary contact of an allergen (e.g. pollen) with the immune system of a potential patient. Following subsequent allergen exposure, allergen-immune complexes formed on mast cells or basophils induce the pathomechanism of allergy, culminating in the release of biological mediators (e.g. histamines or leukotrienes) and the generation of the well-known symptoms of allergy (e.g. rhinitis, asthma, anaphylaxis). More than 40 years ago, IgE antibodies were identified as central mediators of allergic diseases (33), and the first concomitant *in vitro* diagnostic tests for allergy were designed based on the detection of IgE in the serum of allergic patients (34). For the development of these tests, allergenic material is typically immobilised onto a solid phase, such as the well of a micro-plate, a nitrocellulose membrane, or the surface of a glass slide. Subsequently, the patient's serum is incubated in the presence of immobilised allergen(s), and specific IgE is adsorbed and retained at the surface. Bound IgE is then detected by a specific anti-IgE antibody, bearing a label (e.g. fluorescence or HRP) to generate quantitative results.

## Protein microarrays for the profiling of allergen-specific antibodies

The idea of employing protein microarrays to study the presence of disease-related antibodies in blood samples is attractive. Recently, the principle of microarray technology has been adopted to the diagnosis of allergen-specific IgE antibodies (16, 24–26). For several decades, diagnosis of type I allergic diseases was performed by assays based on the principle of the radioallergosorbent test (RAST). RAST was originally introduced in 1967 (34), shortly after the discovery of IgE antibodies (33, 35). Advances in the field of molecular allergology originating in the 1990s have led to the identification of many common disease-eliciting allergens in molecular form. These allergens have been characterized on the DNA and protein levels and many have been produced in recombinant form (36). Eventually, panels of recombinant allergens have been created that comprise the epitope complexity of the corresponding allergen sources, for diagnostic and therapeutic purposes (37–39).

Based on the availability of recombinant allergens, the concept of component resolved diagnosis (CRD) has been introduced (40). Based on CRD, specific immunotherapy (SIT) can be performed using exactly those allergens that have been identified as the disease-eliciting source. An important advan-

tage of such a component-resolved immunotherapy (CRIT) approach is the fact that allergic patients can be treated according to their unique reactivity profile (41). In a number of studies, it has been shown that the combination of recombinant allergens with protein microarray technology adds significant benefits to the microarray-based diagnostic approach (16, 42): (i) the IgE reactivity profile of individual patients to a large number of allergen components can be obtained reliably in a miniaturized format; (ii) microarray-based IgE testing correlates well with state-of-the-art techniques like ELISA, immunoblot or RAST; (iii) allergen panels can be extended or modified easily to develop population-, region- or disease-specific arrays.

### **The benefits and future of microarray-based allergy testing**

In contrast to conventional allergy diagnosis, allergen microarrays are multi-analyte tests that permit the simultaneous analysis of large numbers of allergens in a single step. Also, in comparison with other *in vitro* IgE analytical procedures (e.g. ELISA), the fabrication of allergen microarrays consumes only minute amounts of purified or recombinant proteins and can be done in fairly automated batch procedures. Therefore, the resulting biochip-based applications turn out to be cost-effective and can be assessed in accordance with state-of-the-art requirements for laboratory and *in vitro* diagnostic testing (e.g. reproducibility or accuracy). In addition to the low amounts of biological material required for manufacturing, allergen microarrays typically employ only  $\mu$ l-amounts of clinical samples (such as venous or capillary serum) for the generation of a comprehensive allergen-specific IgE reactivity profile. The latter is especially beneficial in clinical settings where infants and small children have to be analyzed in a routine manner. Above that, the use of different fluorescence labels in combination with individual specific detection antibodies permits the measurement of several analytical parameters (e.g. allergen-specific IgEs and IgGs) in a single assay (43). Differential quantitative monitoring of IgE and IgG antibodies against a large number of allergen molecules will potentially improve the monitoring of immunotherapy when assessing component-based therapeutic applications or potential side effects (e.g. novel sensitisation acquired during immunotherapy). In addition to that, for the development of CRD-based tests, the expandability of the microarray format permits the employment of allergen panels that comprehensively represent the epitope repertoire of the respective natural sources.

From a diagnostic point of view, it is mandatory for allergen microarrays to be validated carefully against the complex background of clinically well-defined phenotypes. In order to achieve efficient clinical studies, it will be important to create allergen microarray panels that contain IgE binding epitope repertoires similar or identical to those occurring in the natural disease-eliciting sources. Prior to providing access to a universal panel of the relevant allergen molecules in sufficient number and quality, hybrid microarrays of selected extracts plus available components may prove most sufficient to create reliable diagnostic results. These »Allergome« microarrays containing the most relevant disease-eliciting sources for screening purposes will represent a primary means to determine region-, population- or disease-specific patterns of allergen-specific IgE reactivity. When employed in epidemiological or clinical studies, these comprehensive screening tools will reveal novel marker allergens for vaccine development, and can be used to identify customised allergen panels to assemble (personalised) diagnostic tests of improved predictive value. The development of specific immunotherapy solutions based on hypoallergenic forms of major allergens (e.g. Bet v 1) is slowly reaching a stage where novel therapeutic applications are being assessed in large clinical trials (44). Especially for this type of treatment, the availability of CRD tests employing the same type of molecules will prove beneficial. However, component-based immunotherapy is still in an early stage of clinical validation, and the first recombinant allergens for therapy are only expected in several years. Nevertheless, allergen microarrays can be used to unveil patterns of major and minor allergen reactivities within a comprehensive portfolio of disease-eliciting sources (e.g. pollen, mites, moulds, animal dander, food) and may therefore exhibit the potential to create individual formulations of extracts for specific immunotherapy already today. Subsequently, allergen microarrays could be employed in a »pharmacoproteomics« approach to monitor the efficacy of specific immunotherapy (e.g. by simultaneously measuring the up or down-regulation of allergen-specific IgE/IgG antibodies in the course of the therapy).

In conclusion, the benefits of protein microarray technology for research and diagnosis of allergic diseases will be fully embraced when the power of massively parallel generation of disease-related antibody binding patterns is exploited in the context of discovery-driven/hypothesis-generating scientific initiatives, directly aiming at an improved understanding of the biological mechanisms underlying allergies, as well as for the development of novel diagnostic and therapeutic applications.

## PROTEINSKI MIKROREDOVI U DIJAGNOZI I ISTRAŽIVANJU ALERGIJSKIH OBOLJENJA

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*Kratak sadržaj:* Mikroredovi proteina predstavljaju jedan od osnovnih postulata moderne proteomike, kako u cilju sistematizacije, tako i radi sistemske analize funkcija, interakcija i količine proteina. Mogućnost analiziranja uzoraka u minijaturnom obliku podstakla je napore za razvijanje novih dijagnostičkih pristupa u lečenju raka, autoimunih oboljenja i alergija. U ovom radu biće razmotrena najnovija dostignuća u razvoju proteinskih mikroredova za profilisanje IgE antitela u dijagnozi alergijskih oboljenja tipa 1.

*Ključne reči:* alergija, biočip, komponentno-razrešiva dijagnoza, IgE, mikrored, molekularna dijagnostika, rekombinantni alergen

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