

Chapter 8

High Throughput Screening of Allergy

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Abstract Allergen avoidance and specific immunotherapy that are based on an accurate diagnosis of allergy have proven to be helpful for the prevention and treatment of allergic diseases. Detection of specific IgE against different allergens in serum or plasma can be used as an important criterion for allergy diagnosis. Conventional methods for the detection of IgE in the clinic are mainly enzyme-linked immunosorbent analysis (ELISA), fluorescence enzyme immunoassays, and radioimmunoassays (RIAs). However, it is difficult to achieve high throughput screening by these conventional methods. Microarrays can test more than 1000 known allergens and are able to resolve reactivities to various allergen components, which is necessary for clinical diagnosis and treatment. Thus, allergen microarrays provide a versatile platform for detecting specific IgE or other types of antibodies against thousands of allergens in a parallel and high throughput way and are currently used in diagnosis of allergy. In this chapter we will provide an overview of allergen microarrays.

Keywords Allergen microarrays · High throughput screening · Allergy diagnosis

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8.1 Introduction: Allergy Diagnosis

Allergen avoidance can effectively reduce the risk of allergies while specific immunotherapy is known to be an effective therapy for allergic diseases. When developing a treatment plan for the allergic patient, typically allergen avoidance or specific immunotherapy, it is necessary to clearly identify what allergens the patient is sensitive to. Therefore, accurate determination of specific allergens is very important for the prevention and treatment of allergic diseases.

At present, the diagnosis of allergy relies mainly on clinical history, in vivo tests, and other laboratory experiments (Hamilton 2010; Maloney et al. 2008; Soares-Weiser et al. 2014). Clinical history is helpful, but is sometimes not definitive or accurate. In vivo tests include provocation tests and skin tests. Provocation tests are the gold standard for allergy diagnosis, but they risk the induction of a severe allergic reaction. Provocation tests must be performed in a controlled situation with good medical first aid, and only implemented after careful and comprehensive assessment of risk and benefit. Skin tests are not always reliable due to a certain number of false positive and negative results and, therefore, must be combined with the clinical history or other tests in order to make an accurate judgment. Allergen-specific IgE testing is the main laboratory test used for allergy diagnosis, and is frequently used in the clinic, but the rate of allergen-specific positivity is not high. Testing of allergen-specific IgE in 200,000 samples of patients with suspected atopic diseases by Peking Union Medical College Hospital in China indicated that the rate of detection of specific IgE for many allergens is low (Wang and Zhang 2012) (see Tables 8.1 and 8.2). Other methods, such as the basophil activation test in vitro, have a relatively high accuracy rate (Sturm et al. 2009), but are infrequently used in clinical practice due to their complexity and the lack of uniform laboratory practices for the detection of histamine or other inflammatory mediators.

Table 8.1 Rate of positive-specific IgE detection to suspected allergens

Suspect allergen	Number tested	Number positive	% positive
d1, <i>Dermatophagoides pteronyssinus</i>	23,662	9078	38.4
d2, <i>Dermatophagoides farinae</i>	20,196	8218	40.7
f24, shrimp	2233	511	22.9
f4, wheat	1809	483	26.7
f1, egg white	1299	382	29.4
f2, milk	1516	381	25.1
f23, crab	1508	213	14.1
f91, mango	492	84	17.1
f3, fish	1024	36	3.5

Referenced from: Wang and Zhang (2012)

Table 8.2 Rate of positive-specific IgE detection to suspected major mixed allergens

Mix inhaled allergen				Mix food allergen			
Suspect allergen	Number tested	Number positive	% positive	Suspect allergen	Number tested	Number positive	% positive
phad	26,728	10,556	39.5	fx5	10,578	1300	12.3
mx2	9527	1537	16.1	fx2	3417	349	10.2
tx6	2983	1209	40.5	fx1	570	86	15.1
tx5	2910	850	29.2	fx3	638	83	13.0
wx7	1198	502	41.9	fx10	1704	76	4.5
tx7	464	126	27.2	fx21	234	41	17.5
gx2	536	118	22.0	fx22	349	26	7.5

Referenced to: Wang and Zhang (2012)

Detection of specific IgE against different allergens in serum or plasma can be used as an important criterion for the clinical diagnosis of allergy (Canonica et al. 2013). The conventional methods for IgE detection are mainly enzyme-linked immunosorbent analysis (ELISA), fluorescence enzyme immunoassays (FEIA), and radioimmunoassays (RIAs). However, these methods have several disadvantages: (1) The execution of these methods is relatively complex; (2) Precious amounts of serum are consumed in one test; (3) The cost can be very high when several allergens require testing; and (4) The detected IgE is usually against whole allergen extracts, not individual allergenic components. Currently, there are more than 1000 known allergens and component-resolved diagnosis based on these allergens is becoming increasingly necessary for improving the accuracy of allergy diagnosis (Treadler and Simon 2013). For all these reasons, it has been difficult to achieve high throughput screening by conventional methods.

Protein microarrays have advantages over conventional-specific IgE testing methods because they can provide high specificity, high sensitivity, high throughput, good repeatability, and require only a small serum sample. Currently, microarrays are being widely used in drug screening (Kumble 2007), proteome research (Templin et al. 2003), and clinical diagnosis (Smith et al. 2004; Spisak and Guttman 2009) as well as for high throughput screening of allergy (Harwanegg and Hiller 2004).

8.2 Allergen Microarrays

Allergen microarrays provide a versatile platform for the detection specific IgE or other types of antibodies against thousands of allergens in a parallel and high throughput way. Currently, they are being used in the diagnosis of aeroallergens, food allergens, and contact allergens such as pollen (Cabauatan et al. 2014), peanut, cow's milk (CM), and egg (Ott et al. 2008), as well as latex (Ebo et al. 2010). See the data collected by Wayne G. Shreffler in Table 8.3 (Shreffler 2011).

Table 8.3 Microarray-based molecular allergen assays

	Allergy type	Allergens	Allergen source	Array used	Patients	Reference
Aeroallergens	Tree/grass pollen	Phl p 1, 2, 5, and 6 and Bet v 1 and 2	Recombinant	In house	51 allergic (history and testing), 11 nonatopic control subjects	Jahn-Schmid et al.
	Ragweed/mugwort	Amb a 1, 5, 6, 8, 9; Art v 1, 3, 4, 5, 6	Natural and recombinant	In house	10 ragweed allergic (history and SPT); 9 mugwort allergic (history and SPT)	Gadermaier et al.
Food allergens	Peanut allergy/tolerance	Ara h 1, 2, 3, and 8; Pru p 3; Bet v 1; Phl p 1, 4, 5b, 7, and 12; CCD	Natural and recombinant	In house	108 peanut-sensitized subjects identified from a healthy cohort of 1085, of which 17 were proved to be allergic and 52 were proved to be tolerant; an additional 12 with peanut allergy were specifically recruited	Nicolau et al.
	Baker's asthma	Six <i>Escherichia coli</i> —expressed wheat proteins selected with sera from patients with baker's asthma; Phl p 1, 5, 7, and 12	Recombinant	In house	22 patients with baker's asthma (history and provocation testing); 32 with wheat allergy (history and provocation)	Constantin et al.

(continued)

Table 8.3 (continued)

	Allergy type	Allergens	Allergen source	Array used	Patients	Reference
	Cow's milk	Bos d 4, 5.0101, 5.0102, 8as1, 8as2, 8β, and 8κ	Natural and recombinant	In house	78 milk-sensitized subjects of varying ages and clinical features	Hochwallner et al.
	Oral allergy syndrome	Mal d 1	Recombinant	Commercial		Ebo et al.
Contact allergens	Latex	Hev b 1, 3, 5, 6, and 8; CCD	Recombinant except nAna c 2 as CCD	Commercial	22 allergic (history and testing); 20 asymptomatic with positive serum IgE levels; 26 healthy control subjects	Ebo et al.
	Latex	Hev b 1, 3, 5, 6, 8, 9, 10, and 11	Recombinant	Commercial, customized	52 adult patients with latex allergy; 50 control subjects with venom allergy	Ott et al.

CCD Cross-reactive carbohydrate determinants
Referenced from: Shreffler (2011)

Ott et al. (2008) investigated 130 infants and children with suspected allergy to CM and hen's egg (HE) using allergen microarrays. They compared the detection result of the allergen microarray method with that obtained by extract-based FEIA and skin prick testing (SPT). Their research found that allergen microarrays show performance characteristics comparable to the current diagnostic tests in the diagnosis of symptomatic CM and HE allergies. The combination of allergen microarray results of α -casein, β -casein, κ -casein, Bosd4, and Bosd5 for CM allergy and Gal d1, Gal d2, and Gal d4 for HE allergy both generated the same or highly similar AUC values as compared to FEIA testing in the diagnosis of both HE and CM allergies. See Tables 8.4 and 8.5.

Hiroshi Kido et al. detect specific IgE against more than 20 natural allergen extracts or purified allergens including milk, egg, wheat, pollen, and house dust using a diamond-like, carbon-coated DLC allergen microarray (Suzuki et al. 2011). They found that there were high correlations (0.822–0.966) between allergen-specific IgE values for these 20 allergens determined by the DLC allergen microarray and the UniCAP system. The DLC allergen microarray showed higher sensitivity than the UniCAP system for the detection of allergen-specific IgE. Lowering the limit of dilution rate in UniCAP system to further dilution at 4–8 folds also could be detected by DLC allergen microarray. When used to detect IgE

Table 8.4 Comparison of assay performance in the diagnosis of CM allergy

Allergen microarray							FEIA	SPT
Allergen	α -casein	β -casein	κ -casein	Bos d 4	Bos d 5	Combination ^a	CM extract	Native CM
AUC	0.6	0.6	0.6	0.7	0.6	0.7	0.7	0.7
Sensitivity (%)	26.2	26.2	38.1	50	23.9	59.5	71.1	60.7
Specificity (%)	97.7	93	88.4	93	95.3	83.7	81.4	48.2
P-value ^b	0.29	0.27	0.43	0.61	0.18	0.5	–	–

^aCombination of all fluorescence intensity values of single CM allergen components

^bP-values of nonparametric paired Wilcoxon tests of FEIA and allergen microarray results with respect to AUC values

Table 8.5 Comparison of assay performance in the diagnosis of HE allergy

Allergen microarray					FEIA	SPT
Allergen	Gal d 1	Gal d2	Gal d4	Combination ^a	HE extract	Native HE
AUC	0.8	0.77	0.6	0.8	0.8	0.8
Sensitivity (%)	57.8	57.8	17.8	53.3	71.1	60.7
Specificity (%)	80.7	80	100	100	86.7	100
<i>P</i> -value ^b	0.61	0.2	0.044	0.98	–	–

^aCombination of all fluorescence intensity values of single HE allergen components

^bP-values of nonparametric-paired Wilcoxon test FEIA and allergen microarray with respect to AUC values

Table 8.6 Comparison of assay sensitivity in detecting allergen-specific IgE in CB and MB

Allergen	CB (1:1 dilution)		MB (1:1 dilution)	
	DLC chip	UniCAP	DLC chip	UniCAP
	(Bue/mL)	(Ua/mL)	(Bue/mL)	(Ua/mL)
Food				
Egg white	30.35	ND ^a	77.41	0.545
	11.02	ND	23.65	ND
	180	ND	134.8	0.96
Ovomucoid	84.89	ND	64.66	ND
	13.3	ND	23.15	ND
	221.4	ND	182.7	1.095
Milk	30.9	ND	61.71	0.54
	18.05	ND	23.15	ND
Inhalant				
Cedar pollen	55.55	ND	90.98	0.96
	21.78	ND	32.2	ND
	54.01	ND	80.76	1.275
<i>Dermatophagoides farinae</i>	47.38	ND	25.53	ND
	63.04	ND	215.6	2.95
	26.48	ND	60.7	ND

CB and MB serums (1:1 dilution) were used for the measurement of allergen-specific IgE levels using the UniCAP system and the DLC chip. Detection limit on the DLC chip: 10 Bue/mL. ^aND, Not detectable by UniCAP assay; <0.35 Ua, arbitrary unit

in cord blood (CB) and maternal blood (MB), they found that IgE was detected by the DLC allergen microarray but not by the UniCAP system (Kamemura et al. 2012) (see Table 8.6). Their work laid a foundation for further research into the relationship between IgE levels in CB and MB with infant allergy.

Lin et al. (2012) successfully improved the accuracy rate in the diagnosis of symptomatic peanut allergy using allergen peptide microarray immunoassays and bioinformatic methods. They found that receiver operating characteristic (ROC) curves for the detection of specific IgE against Ara h 1, Ara h 2, and Ara h 3 alone by allergen peptide microarray were better than the detection of total IgE by the UniCAP method. The ROC curve of the machine learning (SVM) method of analysis, which is based on the combination of IgE results for Ara h 1, Ara h 2, and Ara h 3, was best at predicting peanut allergy (Fig. 8.1). Models created using machine learning methods can predict the outcome of double-blind, placebo-controlled food challenges with high accuracy. Jing Li et al. also used an allergen peptide microarray immunoassay to analyze the epitopes of peanut allergen. They develop a peptide microarray immunoassay using 419 overlapping peptides (15 mers, 3 offset) covering the amino acid sequences of Ara h 1, Ara h 2, and Ara h 3. Specific IgE and IgG4 from patients with symptomatic peanut allergy and from patients who were sensitized but clinically tolerant to peanut were tested and

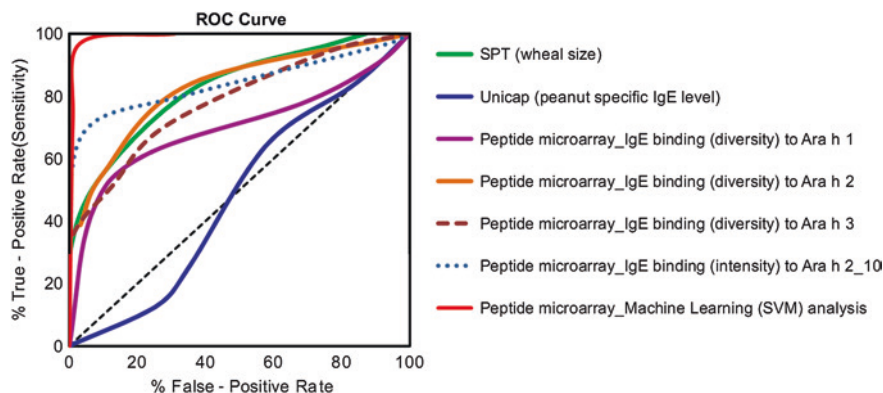


Fig. 8.1 Comparison of the diagnostic performance of different allergy tests and analytical methods in predicting the outcome of double-blind, placebo-controlled food challenges. The area under the *ROC curve* indicates how well a test method can distinguish between two diagnostic groups (peanut allergic vs. peanut tolerant). The *diagonal line* indicates a completely random guess. Both IgE-binding diversity (expressed as the number of positive peptides) and intensity (express as Z score) were measured using peptide microarray (Lin et al. 2012)

compared for binding to these overlapping peptides. Consequently, four peptide biomarkers were found that could better predict peanut allergy.

Allergen microarrays based on detection of specific IgE are not the only tests using patient serum used in allergy diagnosis, but can also be coupled with the basophil activation test. Lin et al. (2007) were the first to couple the allergen microarray method with the basophil activation test in 2007. In their work, the basophilic cell lines KU-812 and RBL-703/21 and human basophils purified from the peripheral blood of healthy donors were resensitized with the serum or IgE preparation to be tested and incubated with manually spotted allergen array chips. Basophil activation, which indicated the effective cross-linking of IgE by allergens, was monitored by upregulation of a basophil activation surface marker (CD63). Their research indicated that coupling the allergen microarray method with the potential functionality and biological activity of a cell-based test is feasible and could result in a new system to detect allergic sensitization. However, the basophil activation test adds more complexity to the diagnosis of allergic disease than using just microarrays based on specific IgE detection, since it is difficult to control the state and response of human basophils.

In summary, with the growing development of allergen microarray technology and its wide application, this method has gradually become an effective tool in high throughput screening for the diagnosis of allergy. It is particularly suitable for patients who require allergen avoidance or specific immunotherapy but have not identified the allergens they are sensitive to and which one is the main cause of their symptoms using traditional diagnostic methods and clinical history. Therefore, allergen microarray technology is a helpful tool to gain information for the prevention and treatment of allergic diseases.

8.3 Preparation of Allergen Microarrays

In order to detect the major allergens using a microarray method, individual allergens are first coated on a solid support. After blocking and washing, the microarray is incubated with the sera of allergic patients followed by the addition of anti-IgE antibodies labeled with either immunofluorescence or enzyme. After washing away unbound anti-IgE antibodies, fluorescence signals are read by a fluorescence scanner (or by confocal microscopy) or photos are taken by charge-coupled device (CCD) camera after chemical color development. By comparing the results with cutoff values and the reference serums, it can be determined which allergens the patients are sensitive to. In order to further improve the sensitivity of the detection system, biotin-avidin or a dendrimer system can also be used to amplify the detection signal. For example, anti-IgE antibodies could be labeled with biotin while streptavidin labeled with fluorescent substances.

The following sections describe the key points of allergen preparation for a microarray system.

8.3.1 *Selecting the Substrates*

Selecting a substrate that can efficiently immobilize proteins on its surface and keep their biological activity is one of the key steps in the development of a protein microarray. A good substrate must meet the following requirements: (1) The substrate should have the reactive groups on its surface to allow interaction with the biomolecules; (2) Maximum binding capacity with biomolecule should be achieved; (3) The substrate should have sufficient stability, including mechanical, physical, and chemical stabilities; and (4) The substrate should have good biological compatibility.

There are two kinds of commonly used substrates:

1. **Filter membrane and gel film**

The filter membrane and gel film are soft substrates that easily adsorb proteins. They can maintain an aqueous environment to preserve protein activity. The biological and chemical properties of the filter membrane are relatively stable. It is helpful for protein immobilization, but has higher background noise and a lower signal-to-noise ratio, and the spotting density is limited. The optical property of the gel film is better than the filter membrane and gel films are good at maintaining protein activity because they have a hydrophilic porous structure. However, the deficiencies of these two types of substrates are that they have high nonspecific adsorption and the possibility of interference between proteins after diffusion.

2. **Enhanced glass slides**

Glass slides have an advantage in that they are inexpensive, have a smooth surface, low detection costs, and stable properties. Modifying the glass surface

is very important for making a protein microarray (Kusnezow et al. 2003). Commonly, the surface of glass slides have been modified with either an amino group, aldehyde group, epoxy group, Mercaptopropyltrimethoxysilane group, polystyrene-based modified surface, and so on (Angenendt 2005). Such substrates immobilize the proteins mainly by covalent bonds or by high-affinity binding between special molecules, which results in tight binding and fewer conformational changes. They have an advantage in maintaining the natural activity of the proteins, as demonstrated by the carboxylated DLC film-coated glass slide that Hiroshi Kido et al. utilized to demonstrate the features of improved glass slides (see Fig. 8.2).

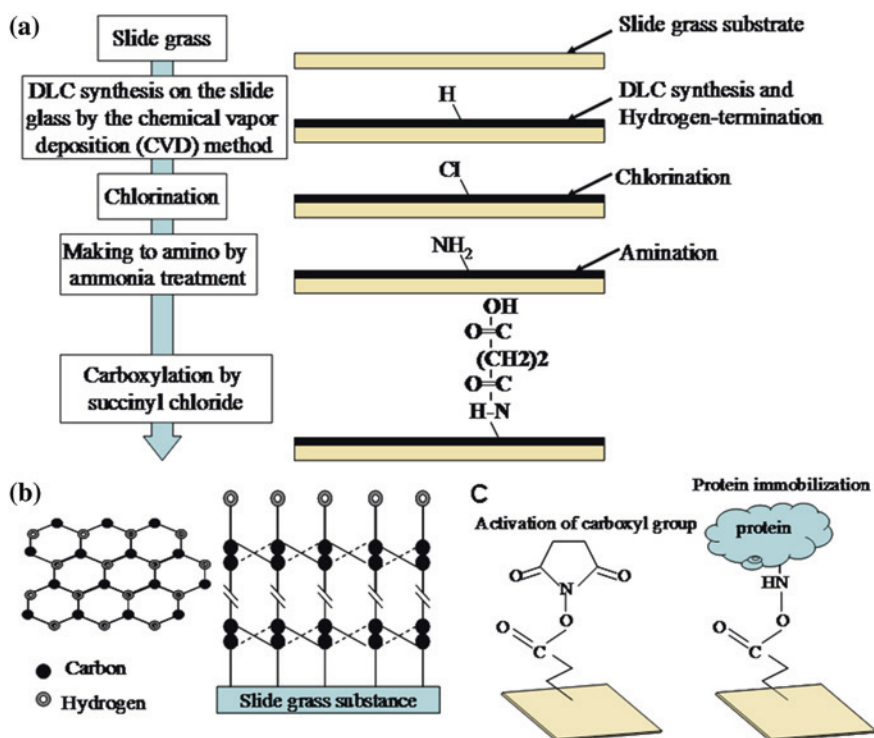


Fig. 8.2 Schematic illustration of the DLC chip, carboxylation, and antigen immobilization. **a** Chemical treatment processes for DLC surface carboxylation. **b** Schematic arrangement of carbon and hydrogen on the DLC chip. **c** Antigen immobilization processes on the DLC chip

8.3.2 Obtaining the Allergens

The allergens used for the preparation of allergen microarrays can be obtained as follows:

1. Allergen extracts

Allergen extracts from dust mites, pollen, mold, cat or dog hair, eggs, milk, crabs, and other allergens are commercially available. However, the components of the extracts are complex and unknown. Using these crude extracts for diagnosis can only roughly determine which allergens the patient is sensitive to with a low accuracy. It does not precisely determine which allergenic components in the extract the patient is sensitive to. Thus, it does not allow for high throughput screening and other clinical requirements necessary for the diagnosis of allergic disease.

2. Native allergen components that have been isolated and purified

Individual allergen components can be obtained by further separating and purifying them from the crude extracts. For example, Asturias, J.A. et al. purified an 18-kDa protein named Pla a 1 from the *P. acerifolia* pollen extract using ion exchange, gel filtration, and reverse-phase chromatography (Asturias et al. 2002). Riecken et al. (2008) purified a natural Ara h 8 in peanut extract using a unique combination of purification steps, including optimized extraction conditions, size exclusion and ion exchange chromatography, and treatment of the interfering contaminants with iodoacetic acid. Natural allergens that have been separated and purified from extracts have certain advantages in allergy diagnosis, but the purification process is tedious and high volume production is difficult.

3. Recombinant allergens

An in vitro expression system can produce abundant amounts of allergens that have advantages such as known molecular weight, stability, quality, high purity, and high volume. Furthermore, their physical properties, chemical properties, and immunological activities are very similar to the native allergens. Therefore, recombinant allergens are an important source for allergens that are used in component-resolved diagnostics and high throughput screening. However, it is still a challenge to obtain some allergens with important posttranslational modifications or that need a fully native tertiary structure (Shreffler 2011).

4. Allergens synthesized by a cell-free system in situ

In situ cell-free protein synthesis is a new technique that uses an exogenous DNA or specific mRNA sequence fixed on the substrate surface as a template to synthesize target proteins (Jackson et al. 2004; Stoevesandt et al. 2011). Tedious steps, such as protein extraction and purification, are avoided and the problem of protein preservation is also easily resolved. With the continued improvements of the in situ cell-free protein synthesis system, the use of a cell-free expression system for preparing allergen microarrays may be a good choice.

8.3.3 Preparation of Detection Probes

Allergens immobilized on solid phase substrates capture the specific IgE, which is followed by the addition of labeled anti-IgE antibodies that detect the allergen-specific IgE. Anti-IgE antibodies can be labeled with fluorescent substances, enzymes, or isotopes. The fluorescently labeled methods are widely used in protein analysis because they have high sensitivity, good selectivity, and a large dynamic range. Enzymatic labeling methods with enzymes such as horseradish peroxidase (HRP) and alkaline phosphatase (AP) are also commonly used. Enzymatic labeling methods have high sensitivity, however, significant variations can arise due to different incubation times (Angenendt 2005). Isotope labeling methods are traditionally one of the most sensitive labeling procedures but are gradually being replaced by other detection methods because of the risks of radioactive contamination.

8.3.4 Detection and Analysis of Signals

In fluorescence-labeled allergen microarrays, a laser scanning confocal microscope can be used to scan for signal, and the mean fluorescence intensity of each point is then analyzed by computer. For enzyme-labeled microarrays, pictures are taken by a CCD camera after color development. The signals are processed by computer to obtain a gray scale for each point. After background signals are subtracted, qualitative and quantitative analysis is performed by comparing the relative amount of signal. Positive and negative and reference serums should always be included in each assay and each sample be tested multiple times to assure reproducibility and confidence in the result.

8.4 Challenges in High Throughput Screening Using Allergen Microarrays

8.4.1 Standardization of Allergens

Allergen components, concentration, and biological activity can all affect the detection of specific IgE. In order to improve the accuracy of IgE detection, close attention should be paid to allergen standardization, and particularly for isolated and purified allergen extracts or allergen components. Different extraction and separation methods cause there to be differences in allergen components and quality that lead to varying abilities to bind with specific IgE. Thus, methods are needed for allergen standardization. The most common allergen standardized method is a competitive IgE-binding inhibition test (e.g., ELISA or RAST),

and it should always be used in the evaluation of the total allergenic activity of an allergen (Grier 2001). Relative potency can be obtained by comparing the target allergen extract with a reference allergen extract. Other biochemical and immunological methods such as SDS-PAGE, immunoelectrophoresis, circular dichroism spectrum analysis, immunoblotting, mass spectrometric analysis, dot blot analysis, proteomic approaches, etc. can all be used in checking and controlling the consistency of composition and activity of the allergen product (Jeong et al. 2011; Larsen and Dreborg 2008). Recombinant allergen proteins also need to be standardized, but it is easier to achieve standardization with recombinant proteins compared to allergen extracts and purified allergen components.

8.4.2 Cross-Reactivity in Allergy Screening

Cross-reactivity is caused by homology of the antigen sequence and structure with other proteins. It often occurs between allergenic molecules in closely related species (e.g., between grass or between mite allergens) or in evolutionarily preserved molecules with similar functions that are present in widely different species and belong to the same protein family (e.g., members of the tropomyosin protein family, such as Der p 10 in house dust mite and Pen m 1 in black tiger shrimp) (Canonica et al. 2013). Allergens that caused cross-reactivity can heavily influence the diagnosis of allergy. For example, birch pollen has cross-reactivity with peanut (Mittag et al. 2004). A patient with birch pollen allergy had high levels of sIgE against birch pollen as well as increased levels of sIgE against peanut, leading to the question of whether the patient was truly sensitive to both of these allergens. Further in depth examination found that the patient's level of sIgE against the main allergenic components of peanut such as Ara h2, Ara h1, and Ara h3 was very low, while only sIgE against Ara h 8 was significantly increased. Given that Ara h 8 can cross-react with birch pollen, this patient was determined to be sensitive only to birch pollen and not also to peanut. Therefore, in order to make an accurate diagnosis of allergic sensitivity, sIgE testing against the main allergenic components as well as to cross-reactive allergens should be performed at the same time (Predki et al. 2005), rather than testing for sIgE against the whole allergen extracts (Pfißner et al. 2012).

8.4.3 High Throughput Screening for Drug Allergy

Drug hypersensitivity reactions (DHRs) are common adverse drug reactions that clinically resemble allergic reactions. When DHRs are mediated by a definite immunological mechanism (either drug-specific antibody or T cell), drug allergies is the preferred term (Demoly et al. 2014). Drugs are capable of inducing all types of immunological reactions, described by Gell and Coombs (Pichler 2003), but

the most common are IgE and T cell-mediated reactions. It is very hard to distinguish between immune mediated drug allergies and nonimmune mediated DHRs just through clinical manifestations and without further laboratory examination. The diagnosis of drug allergies is the same as for the diagnosis of other allergies, such as food allergy, and includes provocation testing, skin testing, and specific antibody detection, as well as the basophil activation test in vitro (Ebo et al. 2011; Romano and Demoly 2007). Detection of sIgE against a drug is helpful for the diagnosis of drug allergy as was shown by Romano A et al. who tested for sIgEs of 70 patients with sensitivity to cephalosporin (demonstrated mostly by anaphylactic shock) using a Sepharose-radioimmunoassay (RIA) method. sIgEs against cephalosporins were detected in 67.1 % of the patients tested (Romano et al. 2005). Manfredi et al. also measured sIgEs of 55 patients with immediate reactions to quinolones, and found positive sIgEs in 54.5.1 % of the patients tested (Manfredi et al. 2004). Protein microarrays have been used in high throughput allergy screening, however, high throughput microarrays for drug allergies based on detection of specific antibody (especially IgE) have not yet been reported. With the development of chip technology and the deepening of drug allergy research, the use of high throughput screening to identify drug allergies and guide safe drug use in the clinic is feasible in the future.

8.4.4 Allergen Microarray Quality Control

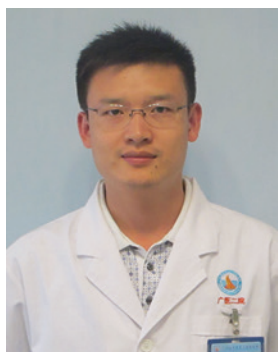
Quality control is an important challenge for allergen microarrays. Without sound quality control, the results produced by experimental microarrays may not be convincing. Several critical steps are involved in the preparation of protein microarrays. The accuracy and repeatability of the results, as well as the quality of the allergen microarrays, depend on many factors such as: (1) Heterogeneity caused by allergen microarray spotting techniques; (2) The quality of the allergen immobilized to the solid phase substrates; (3) Microarray storage conditions-both surface dust aggregation and dewetting impact the quality of the allergen microarray. Also, some allergens are susceptible to degradation, thus, a suitable storage condition is important; (4) Microarray image processing and data acquisition; (5) Other antibody subtypes such as IgG can also affect the detection of allergen-specific IgE. In brief, many factors can interfere with and influence the results of the allergen microarray and cause artificial signals. These interfering signals are not easily identified, therefore, it is necessary to establish criteria that strictly evaluate the results obtained by allergen microarrays.

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Author's Biography



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Prof. Ailin TAO earned his doctorate degree from the State Key Laboratory of Crop Genetic Improvement of Huazhong Agricultural University in 2002, followed by a postdoctoral training at Postdoctoral Station of Basic Medicine in Shantou University Medical College, majoring in allergen proteins. Recent studies of

his lab mainly focus on allergy bioinformatics, allergy and clinical immunology, disease models such as allergic asthma, allergic rhinitis, infection, and inflammation induced by allergy, inflammatory, and protracted diseases caused by antigen or superantigen. He has accumulated some experience in the allergology field including the mechanism on immune tolerance, allergy triggering factors and chronic inflammation pathways, and allergenicity evaluation and modification for food and drugs. He proposed some new concepts such as “Representative Major Allergens”, “Allergenicity Attenuation” of immunotoxin and allergens, “broad-spectrum immunomodulator”, etc. He put forward the theoretical hypothesis of “Balanced Stimulation by Whole Antigens”. He focuses on the diagnosis of allergic disease, and the allergenicity medical evaluation and modification for food and drugs. He constructed the method system for prediction, quantitative assessment, and modification in parallel on allergenicity epitopes, which has been applied for more than 20 cases of allergens. He has developed a bioinformatics software for allergen discriminating, SORTALLER (<http://sortaller.gzhmu.edu.cn>), which performed significantly better than other existing softwares and reached a perfect balance with high specificity (98.4 %) and sensitivity (98.6 %) for discriminating allergenic proteins from several independent datasets of protein sequences of diverse sources, also highlighting with the Matthews correlation coefficients as high as 0.970, fast running speed, and rapidly predicting a batch of amino acid sequences with a single click. The software has been frequently used by researchers from lots of institutions in more than 20 Chinese cities and over 30 countries worldwide, thus becoming the TOP 1 in this field. He has set up an allergen database ALLERGENIA (<http://ALLERGENIA.gzhmu.edu.cn>), which has obvious international advantages in five aspects: wide allergen coverage, nonredundancy, astringency, accuracy, and user-friendly analysis functions.