

The Potential of Allergen Biochips

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Abstract: Immediate-type allergies (type I) allergies to environmental allergens such as plant pollen, pet dander, food, honeybees' and wasps' venom affect around a third of the total population in developed countries. The diseases comprise a broad spectrum from rather mild diseases such as hay fever and skin reactions like urticaria to severe ones such as bronchial asthma, vomiting and diarrhea and finally anaphylactic shock. Type I allergies are caused by an errant immune response leading to the production of allergen-specific IgE.

The usual algorithm for the diagnosis of type I allergies begins with obtaining a detailed patient history and continues with the confirmation by skin tests and/or *in vitro* measurement of IgE. Allergen biochips are a promising new technology for the *in vitro* measurement of specific IgE in type-I allergic patients. In contrast to conventional *in vitro* tools, they consist of multiple allergen components spotted onto a microarray. This allows to perform multiple analyses in a single measurement analysing patient-specific sensitisation patterns, the so called "component resolved diagnosis". This review considers prospects and difficulties with this new technology and also reviews patents related to this field.

Keywords: Allergen biochips, allergen microarray, diagnosis of allergy, *in vitro* diagnosis, type-1 allergy.

INTRODUCTION

Type-I allergy, also known as immediate type allergy is causing considerable morbidity in the general population and affects around 30% of children and young adults [1]. Allergies¹ are caused by a certain subclass of allergen-specific IgE antibodies (sIgE) that are unique to allergic individuals and not found in the general population. sIgE-binding leads to the activation of mast cells that release histamine and other biologically active substances such as leukotrienes. Detection of sIgE in a patient proves the individuals' sensitization.

Clinical allergies evolve in a sequence of diseases coined as "atopic march" [2]. Atopic dermatitis a disease characterized by severe itch, dry skin, and bacterial superinfection - is the first disease appearing at the age of 1 to 4 years. Around a quarter of these children are sensitized to one or more of the most relevant food allergens: milk, egg, wheat, soy, fish, and peanut. The solitary rational treatment is a selective elimination diet that should only be recommended after a thorough diagnostic workup. Non-indicated elimination diets impose significant harm to these small patients by withholding important dietary nutrients. Food-allergy usually disappears in adolescence and only a minority of adults suffers from dietary allergies with the exception of peanut- and fish-allergy that can persist to adulthood.

The situation is different in allergies to aeroallergens. These comprise plant pollen, animal dander, fungal spores,

and house dust mite. Allergic rhinoconjunctivitis also known as hay fever that appear as the 2nd atopic disease in school-children and adolescents significantly reduces intellectual and working capacities as well as quality of life. Early intervention with allergen-specific immunotherapy (also known as desensitization or allergy vaccination) can prevent the transition to allergic bronchial asthma, that occurs in up to 40% of the untreated patients [1]. Allergic bronchial asthma is associated with high morbidity and causes high costs to public health.

Allergy to hymenoptera venom stings (honeybee, yellow-jacket, wasps, hornets and fire-ants) is an own entity independent of other type-I allergies and occurs in around 1% of the general population independent of age [3]. Treatment consists in prescription of self-injectable adrenalin and specific immunotherapy in patients with a history of systemic anaphylactic reactions.

A third entity among type-I allergic diseases are immediate reactions to drugs, mainly penicillins, muscle relaxants and latex during surgical interventions. This is an important issue of public health as e.g. 7.8% of a random sample of the Portuguese population states as having suffered from some adverse reactions to drugs [4]. They comprise broad clinical spectrum from rather mild skin reactions such as urticaria to anaphylactic shock [5].

The mainstay of diagnosing type-I allergy is obtaining a detailed patient's history which gives important information about the type of allergen eliciting the disease and the symptom severity. According to current guidelines, the results of a suggestive history should be confirmed by skin tests and/or *in vitro* measurement of sIgE [1, 6].

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¹Within this article the term "allergy" is used as synonym for type-I allergy.

RECENT DEVELOPMENTS

Naturally occurring allergens, the source of allergen extracts, vary significantly in their composition and allergenicity [7]. Most patients do not raise sIgE to all but only some allergenic components (Table 1). In routine settings, natural allergen extracts are used for skin tests, *in vitro* diagnostics and specific immunotherapy. Currently, allergen components are not available for skin tests or specific immunotherapy.

Depending on the source, allergen extracts contain only one major component, e.g. house cat (*Felis domesticus*) with Fel d 1, others such as grass pollen (*Phleum pratense*) consist of at least 6 relevant components: Phl p 1, 2, 5, 6, 7, and 12 (Table 1) [8]. Quality control in the production process of allergen extracts usually focuses only on selected key components, e.g. Phl p 1 & 2. Hence, skin and *in vitro* testing with allergen extracts might lead to false negative results in a grass-pollen-allergic patient sensitised only to minor grass-pollen components. The correct sensitisation profile is of high importance when choosing the correct composition of allergen-vaccines for specific immunotherapy.

Testing with allergen components instead of testing with natural allergen extracts has been introduced in the last decade into conventional *in vitro* testing, a strategy termed “component resolved diagnosis”. As a major drawback, *in vitro* tests with allergen components have to be performed separately per component. For example, a component resolved diagnosis of a grass pollen allergic patient using Phl p 1, 2, 5, 6, 7 produces 5 times higher costs than testing with the grass pollen extract. What does this mean in real terms? The Austrian social insurance system reimburses private laboratories with 5.56 € per measurement at a maximum of 5 measurements at a time [9], e.g. 5* 5.56 € = 27.80 € for conventional component resolved diagnosis with single measurements vs. 5.56 € with the grass pollen extract. Hence, in the everyday clinical setting a high quality component resolved diagnosis is rarely used due to its high costs.

Based on the concept of DNA microarray technology in the early 1990s, protein microarrays have emerged as a promising approach towards the high-throughput large-scale profiling of protein interactions on a system-wide scale. Recently, the component resolved diagnosis concept has been combined successfully with protein microarray technology to generate allergen-microarrays also known as allergen biochips for the simultaneous monitoring of sIgE antibodies towards a large number of allergy-eliciting molecules [10] Fig. (1). Technical background information is provided in the legend of Fig. (1). Lee Duen and Chin Chuan from Taiwan [11], Reinhard Hiller et. al from Austria [12-15] and Hugh Sampson *et al.* [16, 17] from the USA all disclosed related patents on allergenic components or extracts immobilised on a microarray chip for allergy-related immunoglobulin detection, especially for the quantitative detection of total IgE and allergen-specific immunoglobulins (such as specific IgE, specific IgG, and specific IgM).

CURRENT & FUTURE DEVELOPMENTS

Allergen biochips are a very promising option for the *in vitro* diagnosis of type-I allergy in the near future:

1. Component resolved diagnosis is an important issue for several reasons. Although the world is now referred to as a “global village”, it consists of climatically very distinct regions. Existence of allergenic plants and moulds, pollen grain counts and blossoming periods depend a lot on regional climate conditions. Also, regional alimentation (e.g. fish allergy highly relevant only in maritime countries, high rates of life-threatening peanut allergy restricted to anglo-saxon countries) differs across the world. Furthermore, sensitization patterns differ according to the region of sensitisation with consequences on the clinical course. For example, apple allergy in Northern Europe usually means only a mild oral allergy syndrome caused by cross reaction in birch pollen allergic individuals. In contrast, in southern Europe it is

Table 1. Examples for Clinically Relevant Allergen Components According to www.allergome.org [8]. The List is Growing Day by Day

Allergen name	Birch pollen	Grass pollen	Mugwort pollen	Ragweed pollen	Parietaria pollen	Cow's milk	Cat	House dust mite	Honeybee venom	Yellow jacket venom
Scientific name	<i>Betula verrucosa</i>	<i>Phleum pratense</i>	<i>Artemisia vulgaris</i>	<i>Ambrosia artemisifolia</i>	<i>Parietaria judaica</i>	<i>Bos domesticus</i>	<i>Felis domesticus</i>	<i>Dermatophagoides pteronyssinus</i>	<i>Apis mellifera</i>	<i>Vespula vulgaris</i>
Clinically relevant components	Bet v 1	Phl p 1	Art v 1	Amb a 1	Par j 1	Bos d 4	Fel d 1	Der p 1	Api m 1	Ves v 1
	Bet v 2	Phl p 2	Art v 4		Par j 2	Bos d 5		Der p 2	Api m 2	Ves v 2
		Phl p 5	Art v 5	other	Par j 3	Bos d 6			Api m 3	Ves v 5
		Phl p 6		unclassified					Api m 4	
		Phl p 7		proteins						
		Phl p1 2								

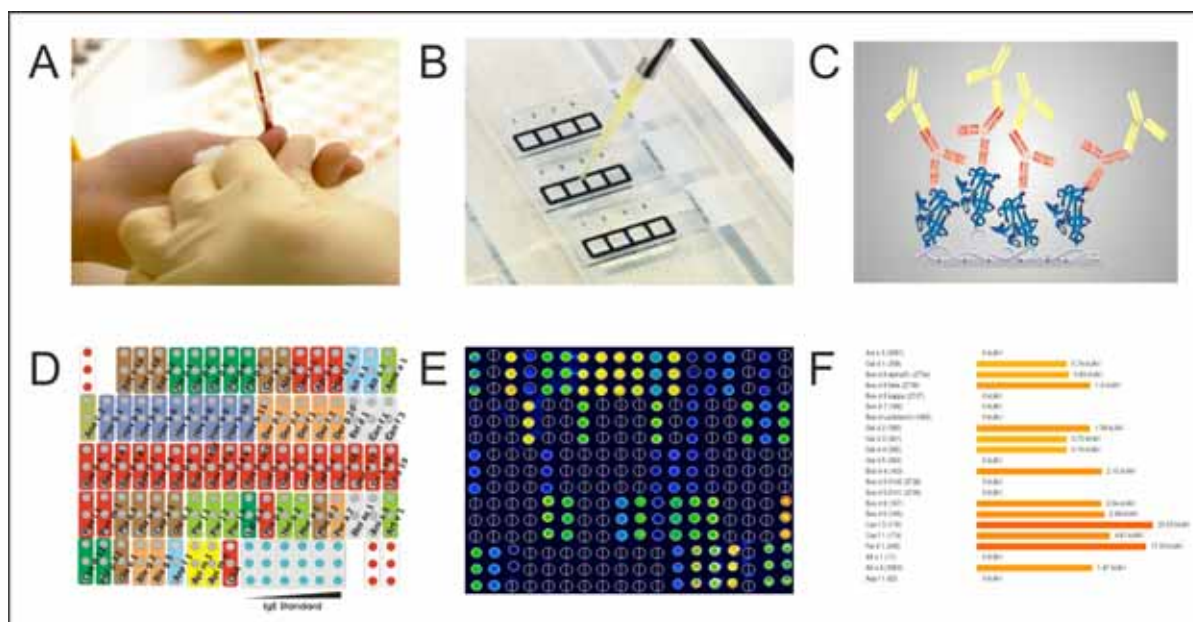


Fig. (1). Allergen microarray design and assay procedure.

A: Minute amounts (20 µl) of capillary or venous blood are required for serum preparation.

B: Incubation of a 4-well chip with 4 individual serum samples (20 µl per serum).

C: Schematic representation of an allergen microarray assay. Allergen molecules (blue) are covalently immobilized onto the chemically modified biochip surface to bind efficiently and in a biologically active manner. Allergens are recognized by serum IgE antibodies (red), which themselves are detected by a fluorescently labeled anti-human IgE antibody (yellow).

D: Schematic representation of the allergen microarray layout. Individual allergens of a single biological source are depicted as circles surrounded by a rectangle bearing the same colour.

E: Allergen microarray false colour sample image. Images are acquired by confocal laser scanning microscopy in a commercial biochip reader. The 16 bit TIFF images display signals between 0 (black) and 65,355 (white).

F: Schematics of result interpretation in coloured bars correlating to the signal intensities obtained from images. Results are expressed in a semi-quantitative way as kUA/L.

The figure is kindly provided by Christian Harwanegg PhD, VBC-GENOMICS, Vienna, Austria.

usually caused by cross reaction to another component in peach-sensitized patients causing severe systemic reactions [18]. In these cases, component resolved diagnosis is the only way of diagnosis because both patient groups will have positive skin test and *in vitro* test results with apple extracts. Another example are patients reacting to virtually all plant allergens in skin tests because of sensitisation to pan-allergens such as profilin that exists in a lot of plant families, e.g. Amb a 8 from ragweed pollen, Ara h 5 from peanut, Bet v 2 from birch pollen, Hev b 8 from latex, Phl p 12 from grass pollen [8, 19]. The puzzle of patients reacting to virtually all plant allergen extracts in the skin tests can be solved by detecting sIgE to profilin with component resolved diagnosis.

2. Having finished phase II [20, 21], some phase III studies are ongoing with the dominant recombinant allergen components of birch pollen (Bet v 1) and grass pollen (Phl p 1, p 2, two variants of p 5: p 5a/p 5b, p 6) for specific immunotherapy. Currently, only allergen extracts are available for skin testing. Hence, the combination with an *in vitro* component resolved diagnosis is a *conditio sine qua non* for the correct selection of the component-based therapy for the individual sensitisation pattern of the patients.

3. Analysis with allergen-biochips is possible with only 15 - 20 µl of body fluid (i.e. serum from venous blood). Conventional *in vitro* methods depend on 100 - 150 µl serum (extracted from around twice the amount of venous blood) per assay. The small amount needed for allergen biochips is especially attractive for *in vitro* diagnosis in small children, where a single drop of blood from the finger tip is enough for performing a complete component resolved diagnosis.

4. Allergen biochips have a high potential for driving allergy research. For example, all allergenic components contain several antibody binding regions, so called "epitopes". Hugh Sampson and colleagues could show that milk allergic patients with sIgE to one epitope outgrew their milk or peanut allergy while those with sIgE to another epitope on the same component kept being allergic for many years [22, 23]. As an example, allergen biochips might ease the identification of such epitopes by screening large patient populations.

Nevertheless, allergen biochips currently also have certain drawbacks:

1. Screening with *in vitro* diagnostic tools *per se* bears the immanent risk of creating clinically irrelevant false results [24]. How comes? 100% correct test systems are

technically impossible. Hence, the medical community has agreed that diagnostic systems with 95% reliability are sufficient for clinical use². This means that when performing 100 tests, 5 of them must be wrong (false positive or false negative). Medical statistics has created two defined terms for addressing this problem of *in vitro* diagnostics:

a: **Sensitivity**: describes the proportion of true patients classified as “allergic”

b: **Specificity**: defines the proportion of subjects correctly classified as “not allergic”³. The concomitant measurement of multiple allergens with a simple measurement has largely increased this sensitivity/specificity problem. The current version of the ISAC[®] allergen-biochip (ImmunoCAP ISAC[®], VBC-Genomics, Vienna, Austria) contains 89 allergen components that are all measured at the same time resulting in 9 false results on each measurement. The main problem is on how to handle this irrelevant/false information as up to now no tool in Medical Statistics has been created for this problem. Skin tests offer no solution because they are - like the *in vitro* measurement of sIgE - sensitive but not very specific. Theoretically, one could perform the specific but insensitive provocation tests in all cases of doubt, e.g. oral food challenges. However, these challenges put the patients at risk and must be restricted to severe and unclear cases. Additionally, challenge tests can only be performed in hospitalised patients which lays big economic burdens on public healthcare systems. Overall, at the moment there is no solution to this problem.

2. This means, that at the moment the clinical expertise of the clinical allergologist is the only way of protecting patients from irrelevant positive results. Hence, the clinical interpretation of the *in vitro* results must be reassured by clinicians with a profound expertise before the test results may be transmitted to each individual patient.
3. Costs: Allergen microarrays are composed of proteins Fig. (1) and the technology behind this must not be mixed up with DNA or RNA microarrays. Proteins are much more difficult to produce and to handle than RNA and DNA and will probably always be more expensive than nucleic acids, even when produced on large scales. Therefore, as already mentioned above the costs are currently the limiting factors when conceiving a broad application of this new technology in everyday clinical practice. Also, the microarray reader is an expensive asset. However, because it is the same machine that can also be used for analysing DNA microarrays, it might

already be existing at certain facilities and an adjustment of the software would be sufficient for the use with allergen biochips.

4. At the moment, only one manufacturer (VBC-Genomics, Vienna, Austria) is able to provide a system that is commercially available for clinical use Fig. (1).

Only a few allergens have been clinically validated yet. A 2006 study showed [25], that of 5 studied allergens, only 4 were suited for the *in vitro* diagnosis of allergy in daily clinical practice when comparing with the industrial quasi standard, the ImmunoCAP[®] system by Phadia AB (Uppsala, Sweden). The problem could and may have already been solved by using better and more allergen components on future versions of the chip. In late 2007, Phadia bought itself into a ¼ of VBC-Genomics. This has already resulted in a new version of the biochip with a higher amount and, importantly, a higher quality of already validated allergen components. Still, all of the new allergen components will need to be validated when used on the allergen microarray.

CONCLUSION

Summing up, allergen biochips currently present a promising technique for the *in vitro* diagnosis of type-I allergy. Component resolved diagnosis is an immanent feature of this technology. Setting higher standards for the *in vitro* diagnosis of type-I allergy by replacing current *in vitro* systems with allergen biochips might increase short-term costs by investing in expensive machinery but may reduce costs on the long run by reducing the number of expensive-to-care asthmatic patients.

CONFLICT OF INTEREST

There is no conflict of interest. I never received speaker's fees or grants from the manufacturers mentioned in the text, hold neither stocks in any of the manufacturers or a patent in this field.

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²Remark: the scientific significance level of $p = 0.05$ is derived from this: $1 - 0.95 = 0.05$.

³No diagnostic tests can be at the same time sensitive and specific. The diagnosis of HIV is a good example on how a sensitive and a specific test are combined to make sure that as many patients as possible are correctly identified as being “HIV positive”. First, a very sensitive 1st screening test – the HIV-ELISA detecting IgG antibody against virus protein – is performed in a population at risk, e.g. sexually active adults. This test will pick out a lot of individuals, but only some of them are really infected with HIV. That's the point where a 2nd specific test such as the Western blot or the HIV-PCR both detecting the virus itself comes in. The diagnosis of “HIV infected” is only valid if both the 1st and the 2nd test are both positive.

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