

Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment¹

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SPECIFIC AIMS

Type I allergy is an immunoglobulin E (IgE) -mediated hypersensitivity disease affecting >25% of the population. Allergy diagnosis is performed by provocation testing and IgE serology using allergen extracts. It defines allergen-containing sources but cannot identify the disease-eliciting allergenic molecules. We have established a panel of purified recombinant and natural allergen molecules that represents the most common allergen sources. These molecules were microarrayed on glass chips in order to develop a miniaturized allergy test that allows the determination and monitoring of allergic patients' IgE reactivity profiles to disease-causing allergens using single measurements and minute amounts of serum.

PRINCIPAL FINDINGS

1. Selection, purification, and characterization of allergen molecules representing important allergen sources

We purified 78 recombinant and 16 natural allergen molecules representative of most important allergen

sources (Fig. 1a). The respiratory allergen sources covered by the purified allergen molecules were tree, grass, weed pollens, mites, animals, fungi, and insects. Allergens from stinging insects, a variety of foods, latex, and an autoallergen were also included (Fig. 1a). Purified allergen components were obtained for most allergens by expression in *Escherichia coli*. Certain allergens were expressed in eukaryotic expression systems or purified from natural sources.

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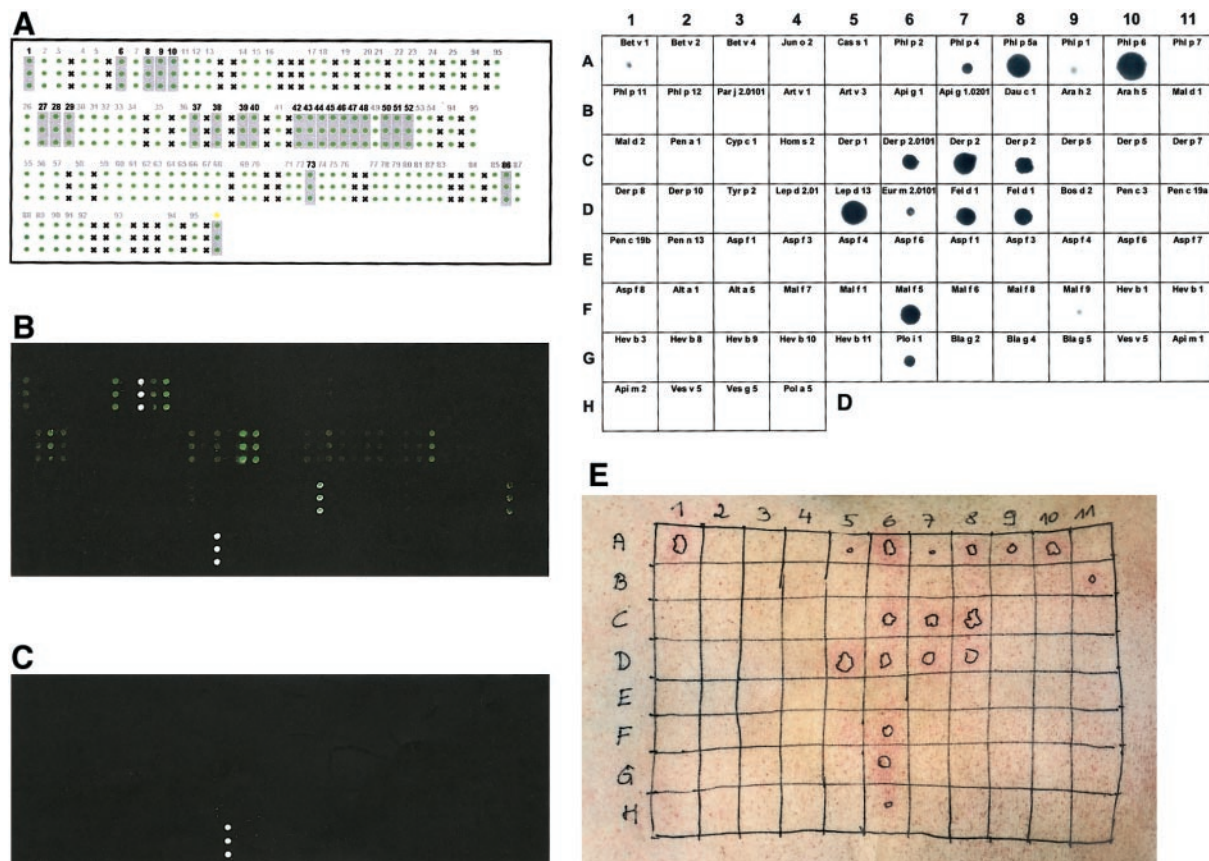


Figure 1. Allergen microarray. *a*) Scheme of environmental allergens (1–93; birch pollen: Bet v 1 [1], Bet v 2 [2], Bet v 4 [3]; juniper pollen: Jun o 2 [4]; chestnut pollen: Cas s 1 [5]; Timothy grass pollen: Phl p 2 [6], Phl p 4 [7], Phl p 5a [8], Phl p 1 [9], Phl p 6 [10], Phl p 7 [11], Phl p 11 [12], Phl p 12 [13]; pellitory pollen: Par j 2 [14]; mugwort pollen: Art v 1 [15], Art v 3 [16]; celery: Apl g 1 [17, 18]; carrot: Dau c 1 [19]; peanut: Ara h 2 [20], Ara h 5 [21]; apple: Mal d 1 [22], Mal d 2 [23]; shrimp: Pen a 1 [24]; carp: Cyp c 1 [25]; mite: Der p 1 [26], Der p 2 [27–29], Der p 5 [30, 31], Der p 7 [32], Der p 8 [33], Der p 10 [34], Tyr p 2 [35], Lep d 2 [36], Lep d 13 [37], Eur m 2 [38]; cat: Fel d 1 [39, 40]; cattle: Bos d 2 [41]; animals: albumins from cat, dog, cattle, mouse, rat, pig, sheep, chicken, rabbit, hamster, horse, pigeon, guinea pig [42–54]; penicillium: Pen c 3 [55], Pen c 19 [56, 57], Pen n 13 [58]; Aspergillus: Asp f 1 [59, 63], Asp f 3 [60, 64], Asp f 4 [61, 65], Asp f 6 [62, 66], Asp f 7 [67], Asp f 8 [68]; Alternaria: Alt a 1 [69], Alt a 5 [70]; yeast: Mal f 1 [72], Mal f 5 [73], Mal f 6 [74], Mal f 7 [71], Mal f 8 [75], Mal f 9 [76]; latex: Hev b 1 [77, 78], Hev b 3 [79], Hev b 8 [80], Hev b 9 [81], Hev b 10 [82], Hev b 11 [83]; moth: Plo i 1 [84]; cockroach Bla g 2 [85], Bla g 4 [86], Bla g 5 [87]; honey bee: Api m 1 [89], Api m 2 [90]; yellow jacket: Ves v 5 [88, 91], Ves g 5 [92]; wasp: Pol a 5 [93]) an autoallergen, Hom s 2 [94], and human serum albumin (negative control: [95]) microarrayed in triplicate. Allergens reactive with serum IgE of a representative patient (*b*) are boxed in gray and denoted with black numbers. Triplicates of buffer dots (x) and labeled anti-IgE (yellow dot) were spotted as negative and positive controls, respectively. *b*, *c*) Scan images obtained with serum of an allergic and nonallergic individual. *d*) IgE reactivity to nitrocellulose-dotted allergens. *e*) Skin reactivity to allergens and histamine (H6).

2. Microarrayed allergen molecules allow determination of allergic patients IgE reactivity profiles

The allergen components were microarrayed in triplicates onto glass slides in groups representing individual sources (Fig. 1a). Microarrayed allergen components were exposed to sera from allergic patients who were sensitized against a variety of allergen sources and who exhibited total serum IgE levels of 28 kU/l–>2000 kU/l (data not shown). Only a small amount of serum (40 µl) from each patient was needed to test IgE reactivity to almost 100 allergen triplicates in a single test; most diagnostic tests now require 50 µl of serum to test a single component or extract.

Figure 1b, c exemplifies overall performance of the allergen array. The polysensitized patient A showed IgE

reactivity to microarrayed allergen components consistent with the clinical sensitization profile (Fig. 1b), whereas the nonatopic persons serum (Fig. 1c) did not react with any of the immobilized allergen components. Both microarrays contained comparable amounts of immobilized labeled anti-human IgE antibodies (Fig. 1b, c). The IgE reactivity profiles determined with the allergen microarrays agreed well with those determined by skin prick testing or with allergen components that had been spotted onto nitrocellulose under conditions of allergen excess (Fig 1d, e).

3. Microarray-based allergy diagnosis reflects the clinical sensitization pattern of patients

To evaluate the allergen microarray, we tested sera from 20 patients allergic to different allergen sources

who showed low (<100 kUA/l), medium (100–500 kUA/l), and high (>500 kUA/l) IgE serum levels. Clinical sensitivity to the different allergen sources was reflected well by the IgE reactivity profile: e.g., patient A with sensitivity to birch, grass pollen, animal dander, mites, and molds reacted with recombinant birch (Bet v 1), grass pollen (Phl p 1, Phl p 2, Phl p 5, Phl p 6), mite (Der p 2, Lep d 13, Eur m 2.0101), insect (Plo i 1), animal (cat: Fel d 1, albumins from various animals), and yeast (Mal f 5) allergens (Fig. 1*a, b*). However, we observed no strict association between the magnitude of wheal reactions in SPT and fluorescence intensity as measured in the microarray-based IgE detection system (data not shown).

CONCLUSIONS AND SIGNIFICANCE

In this study, we present a novel principle for the diagnosis of type I allergy with purified allergens (Fig. 2). Allergen molecules representing important allergen sources were produced as recombinant proteins or purified as natural proteins (Fig. 2, parts 1, 2). The purified allergen molecules were then microarrayed on preactivated glass slides to obtain allergen chips that allow determination of patient IgE reactivity profiles with minute amounts of serum in single tests (Fig. 2, parts 3–5). A major difference between this and other allergy tests is that the microarray contains defined allergen molecules as reactants. The tests used currently are based on allergen extracts consisting of mixtures of allergenic and nonallergenic molecules, and thus do not allow identification of the disease-eliciting molecules.

We found that a single fluorescence-labeled monoclonal anti-human IgE antibody allowed detection of IgE reactivities to immobilized allergens in sera containing high or low total as well as allergen-specific IgE levels (Fig. 1*b*). We obtained comparable results over a range of several serum dilutions (undiluted, 1:2, 1:5, 1:10) that are commonly applied in various established IgE detection systems (RAST, ELISA, immunoblot) (data not shown).

Simultaneous multiallergen testing mimics more closely the natural allergen exposure that occurs in allergic patients to a variety of tiny amounts of allergen molecules.

The recombinant allergen-based microarray allows the detection of specific IgE reactivities to defined allergen components and thus precise identification of disease-eliciting allergens. The IgE reactivity profile can be analyzed in a single and fast determination. Only small amounts of serum are required, making the microarray useful when it is difficult to obtain large amounts of serum (e.g., monitoring onset and development of type I allergy in early childhood).

The glass slides should be able to accommodate several thousands of individual components and per-

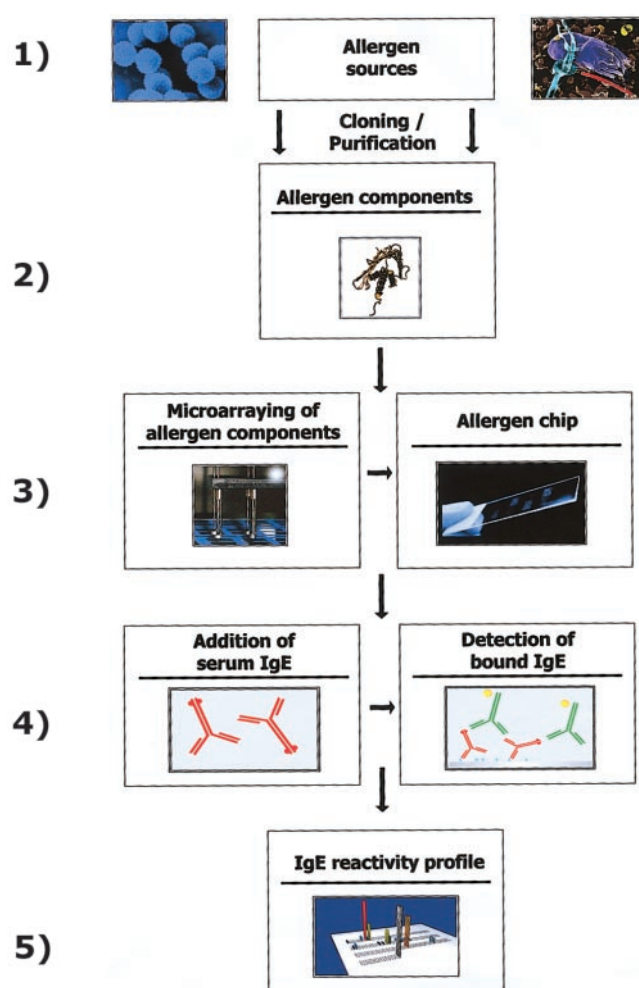


Figure 2. Schematic overview: diagnosis of type I allergy with microarrayed allergen components. (1), (2): Purified allergen molecules obtained from important allergen sources by recombinant DNA technology as recombinant allergens and/or purified natural allergens. (3): Microarraying of purified allergen molecules to obtain allergen chips. (4): Allergen chips incubated with serum IgE and bound IgE antibodies are traced with fluorescence-labeled anti-human IgE antibodies. (5): Patient IgE reactivity profile can be obtained and quantified by scanning fluorescence intensity of the signals.

haps even include allergen epitopes. An advantage of allergen microarrays is that their allergen/epitope repertoire can be expanded and improved according to the availability of newly isolated components.

Using specific antibody probes to immunoglobulin classes or subclasses other than IgE, it will be possible to study allergen and epitope recognition by antibodies that may compete with IgE and to evaluate their possible protective role. It is likely that microarrayed allergen components will fundamentally change the existing practice of allergy diagnosis, prevention, and therapy. Moreover, component-resolved diagnosis based on microarrayed, purified antigens may be applied to autoimmune and infectious diseases. **FJ**