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ORIGINAL ARTICLE

A Photo-immobilized Allergen Microarray for Screening of Allergen-specific IgE

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ABSTRACT

We developed an *in vitro* system to diagnose allergy using an allergen microarray and photo-immobilization technique. Photo-immobilization is useful for preparing the allergen microarray because it does not require specific functional groups of the allergen and because any organic material can be immobilized by a radical reaction induced by photo-irradiation. To prepare the plates, allergen solutions were mixed with polymer and a bis-azidophenyl derivative, a photo-reactive cross-linker, the mixtures were micro-spotted on the plate, and the droplets were dried. The plate was irradiated with an ultraviolet lamp for immobilization. For the assay, human serum was added to the microarray plate. Allergen-specific immunoglobulin E (IgE) adsorbed on the micro-spotted allergen was detected by peroxidase-conjugated anti-IgE antibody. The chemiluminescence intensities of the substrate decomposed by the peroxidase were detected with a sensitive CCD camera. All allergens were immobilized by this method and used to screen allergen-specific IgE.

KEY WORDS

allergen microarray, allergen-specific IgE, allergy diagnosis, chemiluminescence, photo-immobilization

INTRODUCTION

To study allergic reactions, it is important to develop test systems to measure immunoglobulin E (IgE) concentration in serum samples. The first radioallergosorbent test (RAST) to detect allergen-specific IgE in serum was described in 1967.[1] Subsequent tests replaced the radioactive labels used in the RAST with various procedures such as the chromogenic-enzyme immunoassay (EIA) or fluorescence-enzyme immunoassay (FEIA). However, few of these have become routine methods in the diagnosis of allergy in research and clinical practice.[2-7] The most common *in vitro* technique used in the clinical settings is the Pharmacia CAP System (PCS) to measure total and allergen-specific IgE (specific IgE FEIA, Pharmacia, Uppsala, Sweden). Other methods such as the FAST FEIA (MAST Diagnostica, Reinfeld, Germany) and HYTEC EIA (Hycor Biomedicals, Kassel, Germany) are available commercially.[8] Some methods are based on liquid-phase inhibitor assays (e.g., AlaSTAT, DPC Biermann, Los Angeles, CA, USA) or multiallergen-coated nitrocellulose strips (e.g., IgEquick, Teomed AG, Greifensee, Switzerland; CMG Immunodot, Trimedal AG, Brüttisellen, Switzerland).[9,10] The CAP System contains a cellulose polymer densely conjugated with allergen extracts or recombinant allergens.

Although it is possible to measure a multitude of allergen-specific IgEs by immunoassays in the patient's blood, these tests are expensive, time consuming, and some need a high volume of reagents and serum. An increasing number of patients are experiencing immediate-type allergic diseases, such as allergic rhinoconjunctivitis, atopic eczema, and food and drug allergies.[11] It is desirable to develop a fast and economic screening technology to detect allergen-specific IgE in serum samples that allows the simultaneous analysis of hundreds of allergens in a single run. Multiallergen dipstick tests were a first step in the miniaturization and cost savings of such techniques,[10,12] but most could not be run automatically. Microarrays produced with spotting devices are another strategy to miniaturize such tests, which allow

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Table 1 UniCap data of sera (IU/ml)

Allergen	M-14	SIC311276	AHP9580	AHP9549	SIC31181
Japanese cedar	8.79	< 0.34	< 0.34	9.37	30.9
Dermatophagoides pteronyssinus	0.88	36.4	0.63	4.50	12.8
Orchard grass	< 0.34	< 0.34	65.1	99.9	> 100
Cow milk	< 0.34	< 0.34	< 0.34	14.7	37.9
Egg white	< 0.34	0.52	< 0.34	11.4	33.1

The values corresponding to the chemiluminescent spots of Figure 4 are underlined.

proteins to be immobilized in the lower nanoliter range on defined positions on a surface. The first experimental microarray system for allergy diagnosis was reported in 2000,[13] and an allergen microarray based on fluorescence detection was published in 2002.[14] Fall *et al*. reported recently on an application of the parallel affinity sensor array (PASA) technology that automatically performs allergy diagnosis.[2] Purified recombinant and natural allergens and allergen extracts were immobilized on glass slides to detect allergen-specific IgE. However, not all allergens were immobilized by the technique because specific functional groups are needed by the allergens.

We have developed a photo-immobilization method to apply the microarray to various materials including proteins and cells.[15,16] We used this photo-immobilization technique to prepare a microarray of allergens. The advantages of the photo-immobilization method are that it is not limited by functional groups and that it can immobilize any organic material in any organic substrate.

METHODS

REAGENTS AND CHEMICALS

Plates for microarray (polystyrene slides, 2.5 cm × 7.6 cm × 0.5 mm) were cleaned using ethanol with sonication for 15 minutes at room temperature. The washed polystyrene slides were dried and stored. The raw allergen materials, Japanese cedar, orchard grass, *Dermatophagoides pteronyssinus*, cow milk and egg white, were purchased from Allergon (Ängelholm, Sweden). The polyclonal affinity-purified horseradish peroxidase (HRP) -labelled goat anti-human IgE antibody was purchased from Serotec Ltd (Oxford, UK). The ECL Advance Kit for HRP was purchased from Amersham Biosciences UK Ltd (Buckinghamshire, UK). 4,4'-diazido-stylbene-2,2'disulfonic acid, disodium salt (BIS), polyethylene glycol methacrylate (molecular weight, 526 Da), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co (Milwaukee, WI, USA). Sera containing allergen-specific IgE for Japanese cedar (M-14), *Dermatophagoides pteronyssinus* (SIC 311276), orchard grass (AHP9580), cow milk (AHP9549), and egg white (SIC31181) were purchased from Uniglove Research Corp. (Rivera, CA, USA). The reference measurements were performed with the UniCap System (CAP specific IgE-FEIA, Pharmacia, Uppsala, Sweden) and the data are shown in Table 1.

SYNTHESIS OF PEG-350

The polymer matrix carrying polyethylene glycol in the side chains (PEG-350) was prepared as follows. Polyethylene glycol methacrylate (molecular weight 350 Da, 7.0 *g*) was dissolved in ethyl acetate (80 mL) and bubbled with nitrogen gas for 30 seconds. Azobisisobutyronitrile (46.0 mg) was added to the solution, which was then allowed to stand for 6 hours at 60℃. The solution was concentrated and added to diethyl ether. A viscous solid was obtained after stirring. The precipitation procedure was repeated four times and the final precipitate was dried *in vacuo*. The yield was 1.57 *g* (22.4%).

PREPARATION OF ALLERGENS

To prepare the allergen extracts, 5% raw allergen material (w/v) was suspended in 0.05 M phosphate buffer (pH7.4) for 2 hours at 4℃. The supernatant was collected and filtered through a 0.45 μm cellulose acetate membrane (Sartorius, Göttingen, Germany). The supernatant was dialyzed against water for 24 hours and then lyophilized.

PHOTO-IMMOBILIZATION OF ALLERGEN

The principle of immobilization is illustrated in Figure 1. We propose that BIS works as a photo-reactive cross-linker to immobilize the allergen with PEG-350 and that photo-irradiation causes the cross-linking reaction to occur between allergen and allergen, allergen and PEG-350, allergen and the plate surface, and PEG-350 and the plate surface.

The extracted allergens were dissolved in deionized water at various concentrations (0.625−40 mg/mL). The allergen solutions were mixed with an aqueous solution of BIS (0−0.5 mg/mL), and PEG-350 (0.25 mg/mL) at a 2:1:1 volume ratio. The mixtures were micro-spotted (50 nL) with the microarray spotter (PixSis-4500, Cartesian, Irvine, CA, USA) on the plate and the droplets were dried. The microarrayed plate was irradiated with an ultraviolet lamp (300−400 nm, Nippo Electric Co. Ltd. FL15BLB) for 7 minutes. Finally, the allergen-immobilized plates