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Egg white component-resolved diagnosis: Testing of serum ovalbumin-specific IgE by luminescent oxygen channeling immunoassay

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ABSTRACT

Egg allergy is the second most common food allergy in children. Precise measurement of allergen component-specific IgE (sIgE) in serum contributes to molecular diagnosis of egg allergy, i.e. component-resolved diagnosis (CRD). Existing CRD methods, including ImmunoCAP and Immulite, have unavoidable drawbacks such as errors from solid-liquid phase separation and enzyme instability. Therefore, we take egg allergen ovalbumin as an example to establish a homogenous method – indirect luminescent oxygen channeling immunoassay (LOCI) to determine ovalbumin-sIgE precisely. In the indirect LOCI reaction system, ovalbumin-conjugated acceptor nanoparticles were brought close to streptavidin-conjugated donor nanoparticles through antigen-antibody and biotin-streptavidin interactions. The optimal conditions for LOCI were 16.67 μg/mL acceptor-ovalbumin, 1.047 μg/mL biotinylated anti-human IgE antibodies and a 1/20 dilution of serum in a 25-μL reaction volume for a 45-min incubation. We assessed the performance of of this LOCI system, including sensitivity, precision, and anti-interference ability, and compared the diagnostic performance of the LOCI with enzyme-linked immunosorbent assay (ELISA) through a receiver operator characteristic (ROC) analysis. Results revealed that LOCI was superior to ELISA in performance. In summary, we demonstrated that indirect LOCI was an excellent method for precise determination of allergen component-sIgE, contributing to CRD of food allergy.

1. Introduction

Food allergy is a health problem with growing prevalence worldwide [1]. For IgE-mediated food allergies, in vitro testing of allergen-specific IgE (sIgE) in human serum remains an essential diagnostic tool [2]. Conventionally, the crude extract of an allergenic food is used as a known antigen to detect sIgE in serum. However, due to the complex composition of allergenic foods, some low abundance allergen components can be easily lost during the extraction process [3]. Moreover, the allergenic food sources and extracting procedures adopted might be different among manufacturers, which can increase the variability of crude extract quality. These factors may increase the risk of false-negative diagnosis in the clinical assessment of IgE-mediated food allergy, which could lead to missed diagnosis and delayed treatment.

Over the few past decades, with the increasing application of molecular biology techniques to allergen identification, great advances have been made in food allergen profiling. Currently, it is more common to utilize recombinant DNA technology to produce high quality recombinant allergens in large quantities [4]. These improvements facilitate allergen component-specific IgE testing, which is referred to as component-resolved diagnosis (CRD) or molecular diagnosis of food allergy [5,6]. The CRD approach obviates the drawbacks of conventional crude extract-based methods which had low sensitivity, relatively high false-negativity and cross reactivity [7]. Moreover, CRD allows for high-throughput testing of sIgE to a variety of allergen components simultaneously; this feature facilitates the customization of an individualized allergen component panel, which contributed to individualized desensitization [8]. Currently, only two methods have been approved as CRD methods by FDA: ImmunoCAP (Phadia/Thermo Fisher Scientific, Uppsala, Sweden) and Immulite (Siemens Healthcare Diagnostics, Los Angeles, CA, USA) [9]. Both methods have multiple advantages, including the use of enzyme-catalyzed chemiluminescence, the ability to report results both quantitatively (IU/mL IgE) and semi-quantitatively (7 classes from 0 to 6), a wide detection range, excellent analytical reliability, and satisfactory concordance with skin prick test results. However, the dependence on solid-liquid phase separation and

Abbreviations: LOCI, luminescent oxygen channeling immunoassay; CRD, component-resolved diagnosis; sIgE, specific IgE; ROC, receiver operator characteristic; AUC, the area under the curve; OD, Optical density; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; TMB, 3,3′,5,5′-tetramethylbenzidine

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the instability of enzyme activity (e.g., influences from environmental factors such as pH or temperature) are unavoidable drawbacks of these two methods [10,11].

The luminescent oxygen channeling immunoassay (LOCI™) is a homogeneous bead-based method that can resolve the issues mentioned above [12,13]. In a LOCI reaction system, the donor bead can be brought in close proximity (< 200 nm) to the acceptor bead by an antigen-antibody interaction, which enables energy transfer (singlet oxygen) between the two beads and light emission by the acceptor bead [14]. Currently, this technique has been used to detect tumor markers, thyroid hormones, hepatitis B virus antigens and antibodies, interferon-gamma, human insulin, and etc. [15–17]. However, the LOCI technique hasn't been applied to CRD of food allergy so far.

Egg allergy is the second most common food allergy in children. It affects 0.5–2.5% of infants and young children [18]. Children with egg allergy are also at risk of developing other food allergies, including peanut allergy, eczema, and asthma [19]. To date, four major allergens have been well characterized in egg white: ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), and lysozyme (Gal d 4) [20]. Of these allergens, ovalbumin shows the highest abundance [21] and is closely associated with allergic diseases in children [22]. Therefore, in the present study, we, for the first time, used ovalbumin as a representative allergen to develop an indirect LOCI system for detecting serum ovalbumin-sIgE. Without use of enzyme or the trouble of separating free and bound tracers, this LOCI system showed excellent performance in measuring ovalbumin-sIgE in terms of precision, sensitivity and anti-interfering ability. Our work provided a novel and excellent method for allergen component-sIgE determination, which contributed to CRD of food allergy.

2. Materials and methods

2.1. Individual samples and ethics statement

A total of 199 individual human serum samples were collected at Tianjin Children's Hospital from January 1, 2015 to December 31, 2015. Of these 199 serum samples, 86 were diagnosed to have positive reaction to egg white (egg white-sIgE values > 0.35 kU_A/L) and the remaining (n = 113) showed negative reaction.

Our study was conducted in accordance with the current version of the Declaration of Helsinki ethical guidelines as well as the national legal and regulatory requirements. The institutional review board of Tianjin Children's Hospital has approved the study protocol, including patient information and consent form. Our study was also approved by the Ethics Committee of Tianjin Medical University. All subjects provided their written informed consent before being enrolled in the study. All samples we collected are leftover serum samples. All traceable identifiers of the samples were removed to ensure anonymous analysis.

2.2. Enzyme-linked immunosorbent assay

ELISA was used for comparative analysis of its performance with that of LOCI. Briefly, ovalbumin dissolved in PBS (10 μg/μL) was added into 96-well plates, with 150 μL each well. Plates were incubated at 37 °C for 3 h, and then at 4 °C overnight to allow ovalbumin to attach completely. Unattached ovalbumin was removed by washing with 0.05% Tween 20/PBS (PBST). After blocking with 5% skimmed milk in PBST at 4 °C overnight, plates were incubated with diluted serum (1:20 dilution) at 37 °C for 1 h, and subsequently, with horseradish peroxidase (HRP)-labeled anti-human IgE antibodies (1:5000 dilution, A9667, Sigma, USA) at 37 °C for another 1 h. After washing the plate with PBST three times, 3,3′,5,5′-tetramethylbenzidine (TMB) was added to react with HRP in the dark for 15 min. Then, 10% H_2SO_4 was added to terminate the reaction. Optical density (OD) values were measured at 450 nm with a microplate reader (BioTek, USA).

2.3. Optimization of conditions

Forty serum samples (20 clinically positive and 20 clinically negative) were randomly selected to determine the OD values with ELISA. According to the results, we pooled samples to form four groups (n = 5 samples/group), as follows: OD values < 0.3 (negative group); OD values < 0.5 (weak positive group); OD values between 1 and 1.5 (positive group); and OD values > 2 (strong positive group). These four groups of pooled sera were utilized to determine optimal conditions for indirect LOCI.

2.4. Luminescent oxygen channeling immunoassay

2.4.1. Indirect LOCI

The indirect LOCI reaction system comprised four components: ovalbumin-conjugated acceptor nanoparticles, biotinylated anti-human IgE antibody, serum, and streptavidin-conjugated donor nanoparticles. The ovalbumin (Sigma, USA) conjugation to acceptor nanoparticles and the biotin (NHS-LC-Biotin; Thermo, USA) labeling of mouse anti-human IgE antibody (Clone GE-1, Sigma, USA) were performed by Beyond Biotech (Shanghai, China). Streptavidin-conjugated donor nanoparticles are a general purpose reagent in LOCI system whose optimal dose (28 μg/mL) had been titrated by Beyond Biotech (Shanghai, China). The special diluent for donor nanoparticles was also provided by Beyond Biotech (Shanghai, China). The diluent for acceptor nanoparticles was 0.1 M Tris-HCl (pH 8.0) and 3% bovine serum albumin (BSA) in H_2O; the serum diluent contained 0.05 M Tris-HCl (pH 7.95–8.05), 0.15 M NaCl, 2% BSA, 0.05% Proclin, and 0.05% Tween20. The procedure included a 2-step reaction. First, serum was incubated with a mixture of biotinylated anti-human IgE antibody and ovalbumin-conjugated acceptor nanoparticles at 37 °C for 45 min. Then, streptavidin-conjugated donor nanoparticles were added for another 15-min incubation in the dark. When sIgE in the serum interacted with ovalbumin, donor and acceptor nanoparticles were brought together (distance less than 200 nm). This allowed energy transfer from donor to acceptor nanoparticles, generating fluorescent light at 610 nm from acceptor nanoparticles (Fig. 1A). Serum sIgE levels were proportionate to fluorescence intensity, which was evaluated with a High Throughput Chemiluminescence Analyzer (LICA HT, Beyond, China).

2.4.2. Sandwich LOCI

We implemented a sandwich mode of LOCI to quantify IgE (Fig. 1B). In the reaction system, the goat anti-human IgE antibody (polyclonal, sigma, USA), instead of ovalbumin in indirect LOCI system, was conjugated to acceptor nanoparticles; IgE was equivalent to an antigen, and a sandwich complex was formed with two anti-human IgE antibodies. A series of sequential dilutions of a human total IgE standard (WHO IgE reference standard 75/502, [23]) were tested separately, to generate a dose curve of concentration vs. fluorescence intensity. The IgE contents in all dilutions were within the expected sIgE detection range.

2.5. Calibration

Following IgE testing by sandwich LOCI, a standard curve was plotted. The calculated concentrations of sequential IgE dilutions were 3, 1.5, 0.75, 0.375, 0.188, 0.094, 0.047, 0.023, and 0 kU_A/L. From the standard curve of concentration (X) vs. fluorescence intensity (Y), a versatile equation was derived based on the ordinary least squares fit method: $Y = A*X\hat{}B + C*X\hat{}D$.

2.6. Parameters for performance evaluation

LOCI performance was evaluated by determining the sensitivity, assay precision, and anti-interference ability. Sensitivity was expressed as the maximum dilution of positive serum that could be distinguished from negative serum with LOCI. Precision was measured as the inter-