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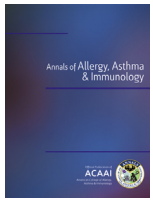

Special consideration is required for the component-resolved diagnosis of egg allergy in infants

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ABSTRACT

Background: There are few reports regarding differences in reactivity to the major egg allergens according to children's age, although component-resolved diagnosis is gradually being used.
Objective: To investigate differences in reactivity to major egg allergens among various age groups of children with egg allergy.
Methods: Twenty-seven patients diagnosed with egg allergy were included. Egg allergy was defined as a convincing history of reproducible symptoms within 2 hours of egg consumption and an egg white–specific IgE level of at least 0.35 kU_A/L. Patients were divided into 3 age groups: younger than 12 months (group A, 7 subjects), 12 to 23 months (group B, 8 subjects), and at least 24 months (group C, 12 subjects). Immunoblotting and enzyme-linked immunosorbent assay investigated IgE reactivity toward ovalbumin, ovomucoid, and ovotransferrin in eggs.
Results: Immunoblotting analysis showed that all patients in group A reacted to ovalbumin, whereas reactions to other proteins were not detected. All patients in group B displayed a reaction to ovalbumin and ovomucoid. IgE binding to ovotransferrin was shown in 3 patients in group B. All patients in group C displayed reactivity to ovalbumin, 5 patients showed a reaction to ovomucoid, and 8 patients displayed a reaction to ovotransferrin. As a patient's age increased, specific IgE binding to ovalbumin and ovotransferrin increased ($P = .011$ and .004).
Conclusion: IgE reactivity to egg allergens differs according to children's ages.

Introduction

Hen's egg is the leading cause of food allergy (FA) in infants and young children, and egg-sensitized children have more severe and persistent dermatitis.[1–3] In addition, egg allergy may precede sensitization to aeroallergens later in life and predict the development of asthma.[4,5] Currently, the standard therapy for managing egg allergy is the strict avoidance of the allergen.[6] However, hen's egg is a versatile food used in the cooking of homemade foods and manufactured food products. Therefore, the dietary avoidance of egg can be challenging. Egg allergy can be sufficiently serious and negatively influence overall quality of life and cause health problems, especially in younger patients.[7]

The double-blinded placebo-controlled food challenge is the gold standard for the diagnosis of FA, but it consumes resources and is associated with a risk for severe anaphylaxis.[6] Alternative tools for the diagnosis of egg allergy include a detailed clinical history, a physical examination, and detection of specific IgE (sIgE) to egg white, although none of these methods is sufficient to meet high predictive values.[6] Recently, molecular diagnostic technologies, such as component-resolved diagnosis or epitope mapping, have been introduced to predict the severity and likelihood of resolution and the presence of egg allergy.[8] The theoretical background of these methods is based on the different clinical significance of pure allergen from natural allergen.[8]

Ovomucoid (OM; Gal d 1, 28 kDa, 11%), ovalbumin (OA; Gal d 2, 45 kDa, 54%), ovotransferrin (OT; Gal d 3, 77 kDa, 12%), and lysozyme (Gal d 4, 14 kDa, 3%) have been identified as major allergens of 23 different glycoproteins in egg white.[9,10] In particular, OM is the immunodominant allergen, although it comprises approximately only 10% of total proteins within the egg white.[9,11] The importance of OM in egg allergy has been explained by the resistance of OM to

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heat and digestive enzymes, reflecting its capacity to stimulate a specific immune response.[10,12] A recent study has reported that the quantitative measurements of OM sIgE could be useful in the diagnosis of egg allergy.[11,13]

Few reports have addressed the differences in reactivity to egg allergens according to a child's age. Moreover, the reactivity to major egg allergens in infants is unclear, although component-resolved diagnosis is being gradually adopted. Understanding the distribution of allergens among different age groups may aid in the development of appropriate diagnosis and management programs for children with FA. Therefore, this study investigated the differences in reactivity to major egg allergens among variously aged children with egg allergy.

Methods

Study Population and Patients' Sera

Twenty-seven patients diagnosed with atopic dermatitis (AD) and egg allergy were included in this study. The diagnosis of AD was based on the criteria of Hanifin and Rajka.[14] The severity of AD was assessed by the Scoring of AD (SCORAD), ranging from 0 to 103.[15] Egg allergy was defined as a convincing history of reproducible symptoms (urticaria, angioedema, respiratory symptoms, or gastrointestinal symptoms) within 2 hours after eating eggs and an egg white sIgE level of at least 0.35 kU_A/L in serum using the UniCAP system (Thermo Fisher Scientific, Waltham, Massachusetts). Patients were divided into 3 groups based on the age at which blood samples were drawn and egg allergy was diagnosed: younger than 12 months (group A; median 8 months, range 5–11 months), 12 to 23 months (group B; median 13 months, range 12–20 months), and at least 24 months (group C; median 33 months, range 24–73 months). Sera from healthy and nonatopic individuals who had never shown symptoms after the ingestion of eggs were used as negative controls.

After obtaining written informed consent, blood samples were collected and sera were frozen at −80°C until use. This study was approved by the institutional review board of the Samsung Medical Center.

Preparation of Egg White Protein Extracts

Egg white protein extract was prepared by adding 10 g of fresh egg white to 90 mL of phosphate buffered saline (PBS) in sterile tubes. The mixture was rotated for 90 minutes at 4°C and then centrifuged at 9,000 rpm for 20 minutes. The supernatants were sterilized through 0.45-μm filters (Carrigtwohill, Cork, Ireland) and lyophilized. Protein concentrations were determined using a microplate reader (Bio-Rad, Hercules, California) with a Bradford protein assay (Bio-Rad). All extracts were stored at −80°C until used.

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis and Immunoblot Analysis

Extracts were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (Tricine System; Invitrogen, Carlsbad, California) according to the manufacturer's protocols. Extracts and proteins were reduced by heating with 4× sodium dodecylsulfate polyacrylamide gel electrophoresis gel-loading buffer (Invitrogen) and loaded into each well of the gel (4%–12% Tris-Glycine gels, 1.0 mm × 10 wells, Novex pre-cast gel; Invitrogen). Precision Plus Protein standards (Bio-Rad) were used as molecular-weight markers to estimate protein size. The separated proteins were transferred from the gel onto a polyvinylidene difluoride membrane using the iBlot Dry Blotting System (Invitrogen). Each membrane was blocked with 2% nonfat dried milk in PBS containing 0.03% Tween-20 (PBS-T) for 1 hour and incubated overnight with the each patient's serum. After washing, the membrane was incubated with biotin-labeled goat antihuman IgE (KPL, Gaithersburg, Maryland), which was diluted 1:2,500 with 2% nonfat dried milk in 0.03% PBS-T for 1 hour. After rinsing, the membrane was incubated with NeutrAvidin-HRP (Pierce Chemical, Rockford, Illinois) for 30 minutes and reacted with the Amersham enhanced chemiluminescence reagent (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) for 2 minutes. The membrane was exposed to a high-performance chemiluminescence film (GE Healthcare Limited, Buckinghamshire, United Kingdom).

IgE Enzyme-Linked Immunosorbent Assay

Extracts and target proteins (OA, OM, and OT; Sigma-Aldrich, St Louis, Missouri) were diluted in coating buffer (0.05 mol/L of carbonate-bicarbonate buffer, pH 9.6) and incubated in microtiter plates overnight at 4°C. After washing with 0.05% PBS-T and blocking with 2% bovine serum albumin (Carl Roth GmbH & Co KG, Karlsruhe, Germany) in 0.05% PBS-T, serum samples were diluted 1:20 (v/v) in blocking buffer and added to the wells. Plates were incubated for 2 hours at room temperature. After rinsing, the wells were incubated for 1 hour with a peroxidase-labeled goat anti-human IgE (Sigma-Aldrich) diluted 1:2,500 (v/v) in blocking buffer. After washing, 100 μL of tetramethylbenzidine substrate (KPL) was added and the optical density (OD) at 595 nm of each well was measured using a VersaMax microplate reader (Molecular Devices, Sunnyvale, California).

Statistical Analysis

The data were analyzed using SPSS 20.0 for Windows (SPSS, Inc, Chicago, Illinois). For the statistical analysis, values greater than 100 kU_A/L were assigned a value of 101 kU_A/L. Sex differences in the 3 separate groups were analyzed by the Fisher exact test. Age, SCORAD, total IgE, and sIgE antibodies against egg white total protein, OA, OM, and OT were compared according to the patients' age using the Kruskal-Wallis test. The level of total IgE was analyzed on a logarithmic scale. A P value less than .05 was considered significant.

Results

Clinical Characteristics of Study Population

The study groups consisted of 7 patients in group A, 8 patients in group B, and 12 patients in group C (Table 1). There was no significant difference in the median SCORAD score among the 3 groups ($P = .088$). No significant differences were found when comparing total IgE and sIgE with egg white among the 3 groups ($P = .165$ and .346, respectively).

IgE Reactivity to Each Egg Allergen as Determined by Immunoblotting

Immunoblotting investigated the IgE reactivity toward OA (45 kDa), OM (28 kDa), and OT (77 kDa) of egg white protein using patients' sera (Fig 1). Overall intensity of IgE binding was enhanced and the number of reactive allergens increased with age. Patients in group A showed a reaction to a protein of approximately 45 kD, which was referred to as OA; however, reactions to other proteins were not detected. In addition, the intensity of IgE binding was weak in group A compared with the other groups, especially in 3 patients (patients 3, 5, and 6). All patients in group B displayed a reaction to proteins of approximately 45 and 37 kD (suggested to be OM). IgE binding to proteins of approximately 75 kD (suggested to be OT) was shown in 3 patients (patients 8, 14, and 15) in group B. All 12 patients in group C displayed reactivity to a protein of approximately 45 kD, 5 patients (patients 17, 23, 25, 26, and 27) showed a reaction to a protein of approximately 37 kD, and 8 patients (patients 17, 18, 20, 22, 23, 24, 26, and 27) displayed a reaction to a protein of approximately 75 kD.