Highly increased levels of IgE antibodies to vaccine components in children with influenza vaccine–associated anaphylaxis

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Background: Influenza vaccines produced in embryonated eggs might pose a risk to patients with egg allergy. However, patients experiencing influenza vaccine–associated anaphylaxis (IVA) do not always have egg allergy. In the 2011-2012 season, an unusually high incidence of IVA was reported in Japan.

Objective: We sought to identify the cause of the increase in anaphylactic events in 2011-2012 in Japan.

Methods: We collected blood specimens from patients with IVA from all areas of Japan. We analyzed 19 patients with confirmed IVA and 25 age-matched control subjects, including 10 with egg allergy who had no adverse events after corresponding vaccination. ELISA was used to measure specific IgE levels to the trivalent vaccines of several manufacturers and hemagglutinin proteins derived from both egg and cell cultures. Antigen-induced basophil activation was evaluated by measuring CD203c expression by means of flow cytometry. Vaccine excipients were also examined for effects on CD203c expression.

Results: None of the patients with IVA had severe egg allergy. Levels of specific IgE antibodies to influenza vaccine antigens, whole-vaccine products from different manufacturers, and hemagglutinin proteins (A H1, H3, and B) derived from both egg and cell cultures were significantly increased in patients with IVA compared with those in control subjects. Influenza vaccine–induced CD203c expression in basophils was also highly enhanced in patients with IVA but not in control subjects. Because IVA was most frequent in patients who received 2-phenoxyethanol (2-PE)–containing vaccine, the effect of this preservative on basophil activation was examined, and the activation was slightly enhanced by 2-PE but not thimerosal.

Conclusions: The 2011-2012 IVA spike in Japan was caused by specific IgE antibodies to influenza vaccine components. Excipients could not be implicated, except for a modest effect of 2-PE. (J Allergy Clin Immunol 2016;137:861-7.)

Key words: *Anaphylaxis, influenza vaccine, hemagglutinin, IgE, basophil activation*

Anaphylaxis after vaccination is a rare but significant problem because it can be fatal if not treated promptly. However, it is sometimes difficult to distinguish anaphylaxis from other adverse reactions, such as the vagal reflex. Therefore proper diagnosis and identification of the causative factor are critical for the management and prophylaxis of anaphylaxis, as well as the prevention of unnecessary contraindications and unnecessary treatments because of an incorrect diagnosis.

Inactivated influenza vaccines are produced by means of inoculation of influenza strains into embryonated hen eggs. This means that there is a risk of anaphylaxis caused by contaminating egg antigens if these vaccines are administered to patients with egg allergy. However, safe influenza vaccine use, even in pediatric patients with severe egg allergy, has been reported,[1-3] which suggests that severe allergic reactions to egg-based influenza vaccines are unlikely. The US Advisory Committee on Immunization Practices recommends that influenza vaccination of patients with severe egg allergy should be performed by a physician with experience in the recognition and management of severe allergic conditions based on the fact that occasional cases of anaphylaxis in patients with egg allergy have been reported.[4]

Although the egg albumin levels in influenza vaccines should be less than 10 μg/mL according to the World Health Organization standard,[5] the level of egg albumin in vaccines produced in Japan is far less than that standard.[6] In the 2011-2012 influenza season one manufacturer (manufacturer A) in Japan reported a significant increase in the incidence of influenza vaccine–associated anaphylaxis (IVA; approximately 1 in 1.4 million doses in normal years but 1 in 0.4 million doses in 2011), although no fatalities were reported. Most of the patients did not have egg allergy contrary to the common belief that contaminating egg

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Abbreviations used
CRTH2: Chemoattractant receptor-homologous molecule express on T_H2 lymphocytes
EA group: Egg allergy group
IVA: Influenza vaccine–associated anaphylaxis
N group: No egg allergy group
2-PE: 2-Phenoxyethanol

protein in influenza vaccines is the main cause of anaphylaxis. The incidence was increased only in the population administered the vaccine produced by manufacturer A and not vaccines from other companies. One of the different ingredients in vaccine A compared with the other vaccines was 2-phenoxyethanol (2-PE), which is used as a preservative, whereas the others used thimerosal. We investigated the cause of these IVA cases by collecting samples from patients from all over Japan and found highly increased influenza vaccine antigen-specific IgE antibodies and positive basophil activation in the patients with IVA but not in control subjects.

METHODS

Subjects

Study subjects consisted of a patient group and 2 control groups. The patient group contained 19 children who experienced IVA in the 2011-2012 season (IVA group). Although the patients lived in different areas of Japan, we contacted the physicians who reported the anaphylactic events and asked them to collaborate on this study. The physicians then arranged for participation of the patients in the study. The diagnosis of IVA was confirmed based on the Brighton Collaboration case definition of anaphylaxis of levels 1 and 2.[7]

We established 2 control groups comprising patients who had undergone influenza vaccination with vaccine A in the same year but had no adverse events. One group consisted of 10 age-matched children with severe egg allergy who were intolerant to eggs at the time of participation in the study and had a history of egg-induced anaphylaxis (egg allergy group [EA group]). The other group consisted of 15 age-matched children with no egg allergy (no egg allergy group [N group], Table I). The subjects' histories of other allergic diseases were confirmed by the attending physicians.

The study protocol was approved by the Ethics Committee of Mie National Hospital. All samples were collected after written informed consent had been obtained from the parents.

Blood samples

EDTA-containing whole blood was collected from the patients and control subjects. The samples were divided into 2 parts: 1 for basophil activation experiments and 1 for obtaining plasma.

Measurement of specific IgE antibody levels

Levels of specific IgE to whole influenza vaccines and the key vaccine components (ie, hemagglutinin proteins) were measured by means of ELISA, as described below.

The investigated vaccines were 4 inactivated trivalent influenza vaccines that had been produced by 4 manufacturers for the 2011-2012 season and 1 produced for the 2010-2011 season in Japan. The vaccine that showed an increased incidence of IVA was produced by manufacturer A for the 2011-2012 season. The vaccines for both the 2011-2012 and 2010-2011 seasons contained hemagglutinin derived from an A/California/7/2009 (H1N1)pdm–like virus, an A/Victoria/210/2009 (H3N2)–like virus and a B/Brisbane/60/2008-like (Victoria lineage) virus, and the corresponding hemagglutinin proteins were separately used as antigens in the assay. In addition to the above hemagglutinin proteins that were produced in embryonated hen eggs, cell culture–derived hemagglutinin proteins devoid of egg protein (Kaketsuken, Kumamoto, Japan) were also used. Other vaccine ingredients, such as preservatives and excipients, namely formaldehyde, phenoxyethanol, and thimerosal, were also tested as antigens. All antigens were dissolved (0.1 mg/mL) in carbonate buffer and placed (0.1 mL per well) in wells of the Nunc-Immuno Plate I (Nunc A_S, Roskilde, Denmark) for 1.5 hours at room temperature. After removal of the supernatants, SuperBlock (Pierce, Rockford, Ill) blocking buffer in PBS was added at 0.15 mL per well and incubated overnight at 4°C. Each well was then washed with PBS-Tween at 0.2 mL per well, and plasma diluted with SuperBlock blocking buffer (1:5) was added at 0.1 mL per well. The plates were incubated overnight at room temperature. After washing with PBS-Tween, biotin-conjugated goat anti-human IgE (1:1,000, 0.1 mL per well; Vector Laboratories, Burlingame, Calif) was added and incubated for 1 hour at room temperature. The plates were washed well, and then streptavidin–horseradish peroxidase (1:5000, 0.1 mL per well; Southern Biotechnology, Birmingham, Ala) was added and incubated for 1 hour at room temperature. The plates were washed well and incubated with a substrate, tetramethylbenzidine solution (ICN Biomedicals, Aurora, Ohio), at 0.1 mL per well for 30 minutes under a light shield. The reaction was stopped by adding 1 N HCl at 0.1 mL per well, and the absorbance at 450 nm was measured with LS-PLATE Manager 2001 (Wako, Osaka, Japan).

Plasma specimens from the 3 patients with anaphylaxis with the highest absorbance measurements were pooled and used as a positive control. Cord blood plasma, which did not contain umbilical IgE, was used as a negative control. A titration curve prepared with the serially diluted and pooled positive plasma was used for quantification of specific IgE levels of the samples with arbitrary units (units per milliliter).

Measurement of CD203c expression on basophils

A commercial kit (Allergenicity Kit; Beckman Coulter, Fullerton, Calif) was used for quantification of basophil CD203c expression. The test was performed according to the manufacturer's instructions. Briefly, EDTA-containing whole blood was incubated with the vaccines and vaccine components at various concentrations for 60 minutes after addition of a sufficient amount of calcium solution to neutralize the chelating capacity of EDTA. Anti-human IgE antibody at 4 μg/mL and PBS served as positive and negative controls, respectively. PC7-conjugated anti-CD3, fluorescein isothiocyanate–conjugated anti–chemoattractant receptor-homologous molecule express on T_H2 lymphocytes (CRTH2), and phycoerythrin-conjugated anti-CD203c antibodies were added during the reaction. The samples were then analyzed on an FC500 flow cytometer (Beckman Coulter). Basophils were detected on the basis of forward side scatter characteristics and negative CD3 and positive CRTH2 results. Upregulation of CD203c on basophils was determined by using a threshold that was defined by the fluorescence of unstimulated cells (negative control) and expressed as the $CD203c^{high}$ percentage.

Statistics

Differences in numeric variables were analyzed with the Mann-Whitney *U* test for unpaired samples and 1-way ANOVA or the Kruskal-Wallis test for more than 2 independent samples, followed by the Dunn multiple comparison test. The χ^2 test was used to examine differences in categorical variables.

RESULTS

Demographic data of the subjects

Table I summarizes the background characteristics of the patients with IVA and the members of the EA and N control groups. A total of 36 pediatric cases of IVA were reported in the 2011-2012 season in Japan, and 19 patients were investigated in this study. Demographics of the rest of the 17 patients who did not participate in the study were also shown. There were no differences in age (in months) or sex among the 4 groups. Egg allergy was identified in only 21% of the IVA group, and no patients with