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

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Mizuho Nagao, MD, PhD,[a] Takao Fujisawa, MD, PhD,[a] Toshiaki Ihara, MD, PhD,[b] and Yoichiro Kino, MSc, PhD[c]
Tsu and Kumamoto, Japan

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

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

TABLE I. Baseline characteristics of patients with IVA and 2 groups of control subjects

	IVA: Investigated in the study	IVA: Not investigated	P value*	EA group	N group	P value†
No.	19	17		10	15	
Age (y), mean ± SD	4.7 ± 1.5	5.0 ± 2.5 y‡	.7856	5.7 ± 2.0	4.8 ± 2.9	.4886§
Range (y)	2-8	1-9		3-10	0-13	
Male sex, no. (%)	14 (74)	11 (64.7)	.7206‖	7 (70)	8 (53)	.4401‖
Egg allergy, no. (%)	4 (21)	3 (17.6)	1.0000‖	10 (100)	0 (0)	<.0001‖
Atopic dermatitis, no. (%)	2 (11)	0 (0)	.4873‖	6 (60)	1 (7)	.0019‖
Asthma, no. (%)	10 (53)	1 (5.9)	.0034‖	10 (100)	5 (33)	.0005‖
History of anaphylaxis (any), no. (%)	0 (0)	0 (0)	NA	10 (100)	0 (0)	<.0001‖
Drug allergy, no. (%)	0 (0)	1 (5.9)	.4722‖	0 (0)	0 (0)	NA
Metal allergy, no. (%)	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA
Latex allergy, no. (%)	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA

NA, Not applicable.

*P values for comparison of the investigated IVA group and the noninvestigated IVA group.

†P values for comparison of all 4 groups.

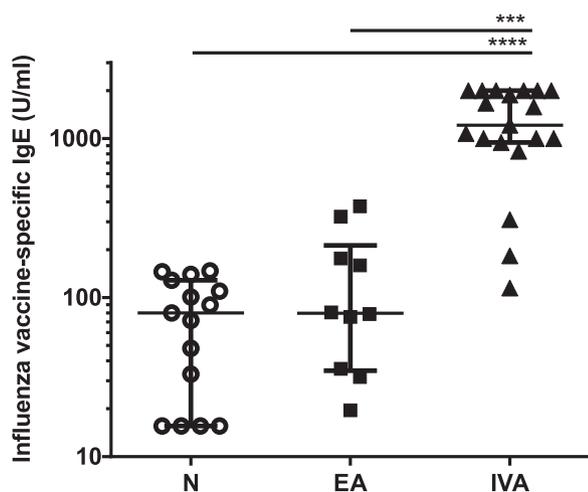‡Unpaired t test.

§One-way ANOVA.

‖χ^2 Test.

FIG 1. Influenza vaccine–specific IgE antibody. *Bars* indicate medians with interquartile ranges. Levels of influenza vaccine–specific IgE were significantly higher in the IVA group than in the N and EA groups. ***P < .005 and ****P < .001, Kruskal-Wallis test, followed by the Dunn multiple comparison test. *Dot plots* lined at 2000 U/mL indicate the values are greater than 2000 U/mL because the upper limit of the assay was 2000 U/mL.

FIG 2. Influenza vaccine–specific IgE to vaccines from different manufacturers. Influenza vaccine–specific IgE levels were measured by means of ELISA, as described in the Methods section. *Solid triangles* indicate subjects in the IVA group, and *open circles* indicate those in the N group. The 2011 product from manufacturer A was associated with an increased incidence of IVA. The IVA and N groups showed no differences in levels of specific IgE to the different vaccines (Kruskal-Wallis test). *Dot plots* lined at 2000 U/mL indicate the values are greater than 2000 U/mL because the upper limit of the assay was 2000 U/mL.

IVA had a history of anaphylaxis. Fifty-three percent of the patients with IVA had bronchial asthma compared with 100% in the EA group and 33% in the N group. In general, the EA group appeared more allergic than the IVA group.

Influenza vaccine–specific IgE antibody

Levels of influenza-specific IgE were significantly higher in the IVA group than in the N and EA groups (Fig 1). The antigen used in the ELISA here was the vaccine that showed an increased incidence of IVA (manufacturer A). Vaccine-specific IgE levels in the IVA and N groups were also measured by using the other vaccine products for 2011-2012 from 3 other manufacturers (ie, B, C, and D) and the product for 2010-2011 from manufacturer A. There were no differences in titers between the products (Fig 2), indicating that the vaccine-specific IgE in the IVA group was specific not only to manufacturer A's vaccine but also to all the influenza vaccines. We then measured specific IgE levels to the
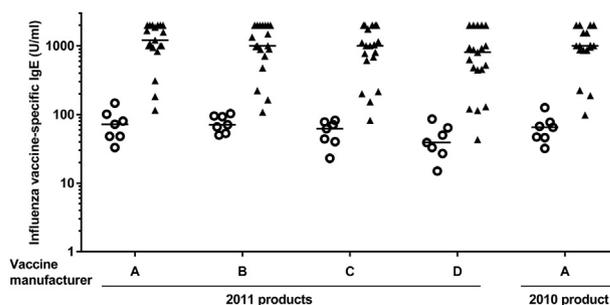
hemagglutinin proteins contained in the vaccines (ie, those from A [H1N1], A [H3N2], and B-like viruses propagated in embryonated eggs [e] and cell culture [c]) and found that the patients with IVA had high levels of hemagglutinin-specific IgE (Fig 3). Excipients used in the vaccines (ie, formaldehyde, 2-PE, and thimerosal) were also used as antigens in the assay, but there was no IgE binding to them (data not shown).

Basophil CD203c expression induced by influenza vaccine

To further investigate the biological significance of influenza vaccine–specific IgE, we examined basophil activation by the vaccines on the basis of CD203c expression. Influenza vaccine–induced expression of CD203c was significantly higher in the IVA group than in the N and EA groups (Fig 4).

2-PE, which was used as a preservative, was the major different ingredient in manufacturer A's vaccine compared with the others. Thimerosal was used as the preservative by manufacturers B, C, and D. On the basis of the above ELISA results that there was no direct IgE-binding activity for the preservatives, we tested the
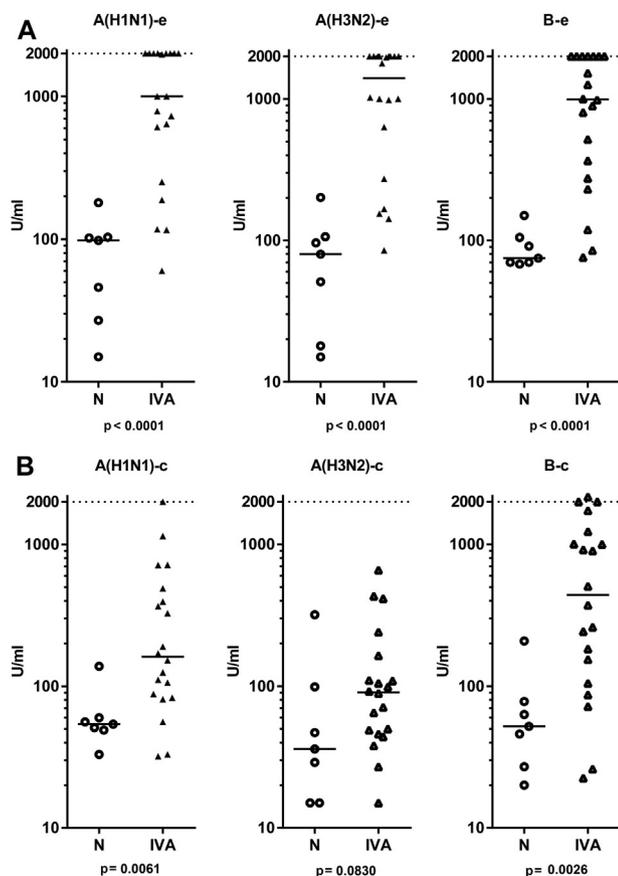
FIG 3. Hemagglutinin protein-specific IgE titers. Hemagglutinin-specific IgE levels were measured by means of ELISA, as described in the Methods section. The antigens used in the assay were embryonated hen egg–derived hemagglutinin proteins abbreviated as A (H1N1)-e, A (H3N2)-e, and B-e **(A)** and cell culture–derived hemagglutinin proteins abbreviated as A (H1N1)-c, A (H3N2)-c, and B-c **(B)**. *Solid triangles* indicate subjects in the IVA group, and *open circles* indicate those in the N group. Differences between the IVA and N groups were analyzed by using the Kruskal-Wallis test. *P* values are shown at the bottom of each graph. *Dot plots* lined at 2000 U/mL indicate the values are greater than 2000 U/mL because the upper limit of the assay was 2000 U/mL.
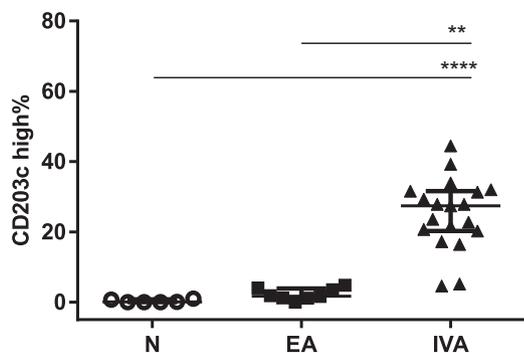

FIG 4. Basophil CD203c expression induced by influenza vaccine. Peripheral blood from the subjects was incubated with the influenza vaccine of manufacturer A at a dilution of 1:333. *Bars* indicate medians with interquartile ranges. Levels of influenza vaccine–induced expression of CD203c in basophils were significantly higher in the IVA group than in the N and EA groups. **$P < .01$ and ****$P < .001$, Kruskal-Wallis test, followed by the Dunn multiple comparison test.

effects of 2-PE and thimerosal on the vaccine-induced expression levels of CD203c. We used vaccine devoid of both preservatives and added them at the same concentration as the final product. This experiment was planned and performed after the initial basophil activation experiments shown in Fig 4. For that reason, we considered the possibility that basophil reactivity to these antigens might already have decreased if blood sampling was performed too long after the anaphylactic event, and we selected 8 cases for whom less than 3 months had passed since the event. Thimerosal showed no effect on basophil activation (data not shown), whereas 2-PE significantly enhanced CD203c expression in the higher vaccine dilutions of 1:1000 and 1:3333 (Fig 5).

Influenza vaccination of 2011-2012 patients with IVA in the 2012-2013 season

One contraindication for influenza vaccination is a history of anaphylaxis caused by any component of the vaccine. However, if influenza vaccine that does not contain phenoxyethanol might be safe, patients can avoid an "unnecessary" contraindication and be eligible for vaccination. With approval from the ethics committee and informed consent from the patients, we vaccinated 3 of the patients with IVA in the 2012-2013 season with manufacturer A's 2012-2013 vaccine, which contained thimerosal rather than 2-PE, for 2 vaccines and manufacturer B's vaccine for the third. None of those patients experienced anaphylaxis, but 2 experienced rather extensive local swelling. One of those patients was not administered the second vaccination. The second underwent the second vaccination after premedication with a nonsedating antihistamine, which resulted in reduced swelling. The third patient had a mild cough after the first vaccination, which resolved spontaneously without treatment. He showed no symptoms after the second vaccination (Table II).

DISCUSSION

After a spike in IVA in 2011 in Japan, we promptly collected blood samples from patients in collaboration with the vaccine manufacturer and attending physicians in all areas of Japan. Our aim was to determine the cause of the increase in this life-threatening event. We were able to enroll 19 of the patients who had experienced this rare allergic event in 2011. Because IVA has been known to be related to severe egg allergy, we recruited 2 groups of age-matched control subjects who had been administered the same vaccine safely in the 2011 season: one group had severe egg allergy and the other group had no egg allergy. We found that the patients with IVA had increased levels of IgE antibodies to the influenza vaccine components, and their basophils were significantly activated by the vaccine. Only 21% of the patients with IVA had egg allergy, but it was not severe, and they had no history of anaphylaxis. These findings strongly suggest that the IVA events were caused by allergy to some component of the influenza vaccine but not to its egg component.

The egg albumin level of influenza vaccines produced in Japan is less than 0.8 ng/mL,[6] which is much less than the World Health Organization standard.[5] Most inactivated trivalent vaccines distributed in other countries, such as the United States and United Kingdom, also contain very low amounts of egg proteins,[8] and recent reports stated that the vaccines are safe even for most patients with severe egg allergy.[1-3] However, anaphylaxis after
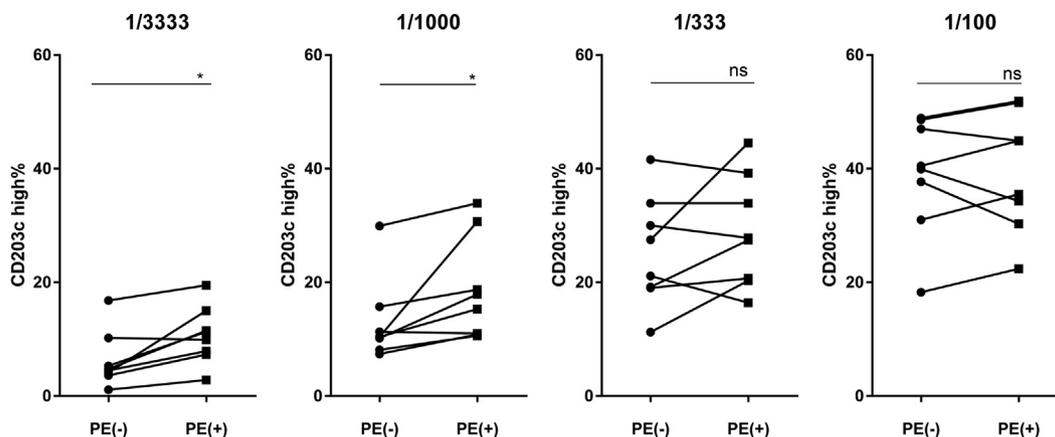
FIG 5. Effect of 2-PE on influenza vaccine–induced expression of CD203c on basophils from patients with IVA. Influenza vaccine devoid of 2-PE was serially diluted to the indicated concentrations, and whole blood from 8 patients with IVA was incubated in the presence and absence of 2-PE at a concentration found in the vaccine product. CD203c expression was significantly enhanced by 2-PE at vaccine dilutions of 1:1000 and 1:3333. *$P < .05$. *ns*, Not significant.

TABLE II. Revaccination of patients with IVA the following year

Patient no.	Age (y)	Sex	Manufacturer	Adverse events	
				Systemic	**Local**
1	3	F	B	None	Swelling developed at the injection site a few hours after vaccination and grew to reach over the elbow on the following day. The second vaccination was canceled.
2	4	M	A	None	Swelling spread to the elbow after 5-6 h. The second vaccination was performed after premedication with a nonsedating antihistamine, and the swelling was smaller in size than after the first vaccination.
3	7	M	A	Mild coughing was observed 30 min after the first vaccination. This resolved spontaneously within 50 min. No symptoms were observed after the second vaccination.	None

F, Female; *M*, male.

influenza vaccination still occurs, although the incidence is very low. Recently, the Vaccine Adverse Event Reporting System in the United States received reports of 12 cases of allergic reactions after immunization with an egg-free trivalent recombinant hemagglutinin influenza vaccine (RIV3).[9] The patients had a history of allergies (particularly to eggs) or previous reactions to other influenza vaccines, which probably led to their being administered RIV3. All the symptoms reported were immediate-type symptoms, which is consistent with IgE-mediated reactions. Direct identification of the symptom-eliciting IgE was not performed.[9] In the present study we showed that patients with IVA had high levels of hemagglutinin-specific IgE, and their basophils were actually activated by the offending vaccine.

Other vaccine components and excipients have also been reported as possible causes of anaphylaxis. Gelatin, an excipient, was once a major cause of anaphylaxis related to measles and other vaccines.[10,11] Although it is still used in a yellow fever vaccine, gelatin has been eliminated from other vaccines produced in Japan, and thus the incidence of gelatin-related anaphylaxis has been significantly reduced.[11] The Sabin vaccine, an oral polio vaccine, contains cow's milk protein and was reported to cause an immediate allergic reaction in children with milk allergy.[12]

Allergic reactions to yeast-derived hepatitis B vaccine[13,14] and allergic reactions against the rubber caps of vaccine vials in patients with latex allergy[15] have also been reported. Influenza vaccines do not contain yeast. Our patients with IVA did not report having latex allergy, and they had not experienced allergic reactions with other vaccines in vials with a rubber cap.

The strengths of our study are that, in spite of the low incidence of IVA, we were able to recruit a relatively large number of patients with IVA in the same season, and we were also able to promptly develop the IgE and basophil activation assays. In Japan physicians are required to report all adverse events after vaccination to the government's Pharmaceuticals and Medical Devices Agency. The Pharmaceuticals and Medical Devices Agency then provides vaccine manufacturers the pertinent information (without patient identification) so that they can take measures as quickly as possible. In 2011, manufacturer A realized that the reports of anaphylaxis with their products were very high, about 3- to 5-fold the average for other years. Because we had treated several early cases in our area and had started to investigate the cause, the company helped us to collect samples from physicians from all over Japan who had treated some of those patients with IVA. In the end, more than half of the

concerned patients with IVA (19/36 [53%]) were able to be enrolled in this study.

Our study is not the first to investigate IgE antibody to influenza vaccines and their components, but it is the first detailed study to demonstrate a strong association between influenza vaccine–specific IgE and IVA. Smith-Norowitz et al[16,17] reported, from the viewpoint of protective immunity, increased IgE antibody levels to influenza virus after vaccination and after infection. Their study cohorts were very small, and they showed no evidence of IgE function, either protective or allergic. In Canada there was a large increase in anaphylactic and allergy-like reactions to the AS03-adjuvanted monovalent pandemic H1N1 vaccine in 2009.[18] The investigators used skin prick tests with full-strength products to identify IgE-mediated reactions, but only 4% of patients and 3% of control subjects had positive responses. They concluded that IgE-mediated allergies to the pandemic vaccine were rare and that the reactions were either coincidental or attributable to other immunologic reactions to the vaccine.[18] We also performed skin prick tests with full-strength vaccine on 3 of our patients with IVA and 10 control subjects and found that all the patients with IVA had positive responses, whereas all the control subjects, including those with egg allergy, had negative responses. Our enrolled patients with IVA lived throughout Japan, some remote from us, and the collaborating physicians could send us blood samples only after obtaining informed consent. Thus we were not able to perform skin tests on all the patients, and we have not presented the findings in the Results section. A major difference between our study and reports from the United States[9] and Canada[18] was the age of the patients. Our patients were mostly male children around 5 years old, whereas the North American patients with anaphylaxis were mainly middle-aged women. The pathogenesis might differ.

It is unclear why only the vaccine product of manufacturer A and not those of other manufacturers caused an increase in IVA events compared with average years. Because 2-PE was the only ingredient different from the other products, we investigated the effect of 2-PE on influenza vaccine–induced basophil activation and found that it enhanced the activation in the higher vaccine dilutions of 1:1000 and 1:3333. 2-PE is thought to be a safe preservative, and it is commonly used in, for example, cosmetics, ophthalmic solutions, and foods. Several cases of contact dermatitis caused by 2-PE have been reported.[19-21] However, no specific IgE antibody was detected.[21] Although there is a report of anaphylaxis caused by 2-PE, the anaphylaxis was induced by a topical product.[22] Use of 2-PE in vaccine products has gradually increased, replacing thimerosal.[23] No previous report of 2-PE in vaccines as the cause of anaphylaxis have been reported. Here we show the possibility of enhancement of IgE-mediated reactions by 2-PE in vaccine-sensitized subjects, but there have been no reports suggesting this with other types of vaccine containing 2-PE. It might be that an adverse interaction between 2-PE and vaccine components occurs only in the case of influenza vaccines.

In a separate experiment by manufacturer A on the physicochemical properties of the vaccine, it was observed that the particle sizes of the vaccine components increased slightly during incubation with 2-PE, raising the possibility that larger particles might have promoted cross-linking of IgE antibodies (personal communications). Based on the results of our study and their own investigations, manufacturer A stopped producing the 2-PE–containing influenza vaccine and replaced it with a thimerosal-containing vaccine for the 2012-2013 season; the incidence of anaphylaxis returned to the historical level.

Shortcomings of the present study are that we did not prove a direct causal relationship between influenza-specific IgE and IVA and that we did not elucidate the detailed mechanisms underlying IVA. However, challenge tests cannot be ethically performed. Our demonstration of influenza vaccine–specific IgE and vaccine-induced basophil activation in patients with IVA might suffice to prove the relationship. We also examined lymphocyte proliferation in response to the vaccine in some of the patients and control subjects but found no differences between the groups (data not shown). Our results of screening for IgG antibodies in the hemagglutinin inhibition test also did not differ between the patients and control subjects (data not shown). Further investigation of other possible immunologic mechanisms might be necessary to understand the pathology of IVA.

In conclusion, for the first time, we clearly demonstrated significantly increased influenza vaccine–specific IgE and vaccine-induced basophil activation in patients with IVA who were included in the IVA spike in 2011-2012 in Japan. The patients were all children (average age, 62 months), mostly male, and with no severe egg allergy. It is noteworthy that phenoxyethanol, which is used as a preservative in the causative influenza vaccine, might have enhanced the allergic reaction. No other risk factors were found, and we believe there is no basis for adding any new contraindications for influenza vaccination at this point. However, clinicians need to be alert to the possible presence of IgE to vaccine components if there is occurrence of a rare immunologic complication of influenza vaccination.

Clinical implications: Anaphylaxis after administration of inactivated trivalent influenza vaccines might be caused by specific IgE to an influenza vaccine component and not to contaminating egg protein.

REFERENCES

1. Forsdahl BA. Reactions of Norwegian children with severe egg allergy to an egg-containing influenza A (H1N1) vaccine: a retrospective audit. BMJ Open 2012;2: e000186.
2. Des Roches A, Paradis L, Gagnon R, Lemire C, Bégin P, Carr S, et al. Egg-allergic patients can be safely vaccinated against influenza. J Allergy Clin Immunol 2012; 130:1213-6.e1.

3. Greenhawt MJ, Spergel JM, Rank MA, Green TD, Mansoor D, Sharma H, et al. Safe administration of the seasonal trivalent influenza vaccine to children with severe egg allergy. Ann Allergy Asthma Immunol 2012;109:426-30.

4. Grohskopf LA, Olsen SJ, Sokolow LZ, Bresee JS, Cox NJ, Broder KR, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP)—United States, 2014-15 influenza season. MMWR Morb Mortal Wkly Rep 2014;63:691-7.

5. World Health Organization. Recommendations for the production and control of influenza vaccine (inactivated). WHO Technical Report Series 2005;927:99-134.

6. Li JT, Rank MA, Squillace DL, Kita H. Ovalbumin content of influenza vaccines. J Allergy Clin Immunol 2010;125:1412-3, author reply 1413-4.

7. Ruggeberg JU, Gold MS, Bayas JM, Blum MD, Bonhoeffer J, Friedlander S, et al. Anaphylaxis: case definition and guidelines for data collection, analysis, and presentation of immunization safety data. Vaccine 2007;25:5675-84.

8. Erlewyn-Lajeunesse M, Lucas JS, Warner JO. Influenza immunization in egg allergy: an update for the 2011-2012 season. Clin Exp Allergy 2011;41: 1367-70.

9. Woo EJ. Allergic reactions after egg-free recombinant influenza vaccine: reports to the US vaccine adverse event reporting system. Clin Infect Dis 2015;60:777-80.

10. Sakaguchi M, Nakayama T, Inouye S. Food allergy to gelatin in children with systemic immediate-type reactions, including anaphylaxis, to vaccines. J Allergy Clin Immunol 1996;98:1058-61.

11. Nakayama T, Aizawa C. Change in gelatin content of vaccines associated with reduction in reports of allergic reactions. J Allergy Clin Immunol 2000;106:591-2.

12. Parisi CA, Smaldini PL, Gervasoni ME, Maspero JF, Docena GH. Hypersensitivity reactions to the Sabin vaccine in children with cow's milk allergy. Clin Exp Allergy 2013;43:249-54.

13. Grotto I, Mandel Y, Ephros M, Ashkenazi I, Shemer J. Major adverse reactions to yeast-derived hepatitis B vaccines—a review. Vaccine 1998;16:329-34.

14. Barbaud A, Deschildre A, Waton J, Raison-Peyron N, Trechot P. Hypersensitivity and vaccines: an update. Eur J Dermatol 2013;23:135-41.

15. Russell M, Pool V, Kelso JM, Tomazic-Jezic VJ. Vaccination of persons allergic to latex: a review of safety data in the Vaccine Adverse Event Reporting System (VAERS). Vaccine 2004;23:664-7.

16. Smith-Norowitz TA, Wong D, Kusonruksa M, Norowitz KB, Joks R, Durkin HG, et al. Long term persistence of IgE anti-influenza virus antibodies in pediatric and adult serum post vaccination with influenza virus vaccine. Int J Med Sci 2011;8: 239-44.

17. Smith-Norowitz TA, Kusonruksa M, Wong D, Norowitz MM, Joks R, Durkin HG, et al. Long-term persistence of IgE anti-influenza A HIN1 virus antibodies in serum of children and adults following influenza A vaccination with subsequent H1N1 infection: a case study. J Inflamm Res 2012;5: 111-6.

18. Rouleau I, De Serres G, Drolet JP, Banerjee D, Lemire C, Moore A, et al. Allergic symptoms after pandemic influenza vaccination rarely mediated by vaccine-specific IgE. J Allergy Clin Immunol 2012;130:1423-6.

19. Birnie AJ, English JS. 2-phenoxyethanol-induced contact urticaria. Contact Dermatitis 2006;54:349.

20. Hernandez B, Ortiz-Frutos FJ, Garcia M, Palencia S, Garcia MC, Iglesias L. Contact urticaria from 2-phenoxyethanol. Contact Dermatitis 2002;47:54.

21. Bohn S, Bircher AJ. Phenoxyethanol-induced urticaria. Allergy 2001;56: 922-3.

22. Nunez Orjales R, Carballas Vazquez C, Carballada Gonzalez F, Boquete Paris M. 2-phenoxyethanol-induced contact urticaria and anaphylaxis. J Investig Allergol Clin Immunol 2010;20:354-5.

23. Centers for Disease Control and Prevention. Frequently asked questions about thimerosal (ethylmercury). http://www.cdc.gov/vaccinesafety/Concerns/thimerosal/thimerosal_faqs.html#e. Accessed August 21, 2015.