

## Serological examination of IgE- and IgG-specific antibodies to egg protein during influenza virus immunization

BY N. YAMANE AND H. UEMURA

*Department of Laboratory Medicine, Kumamoto University Medical School, 1-1-1, Honjo, Kumamoto 860, Japan*

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### SUMMARY

The concentrations of serum IgE (PRIST) and IgE- and IgG-specific antibodies to egg protein were determined in paired sera taken from students who had received influenza virus vaccine. Although persons who gave a history of allergy to egg or to chicken feathers were excluded, 10-16% of vaccinees possessed higher titres of serum IgE and IgE-specific antibody (RAST) to egg white (F1) allergen before vaccination. The titres of IgG-specific antibody to egg protein (ovalbumin and ovomucoid antigens) were negligible, and did not show any significant response after vaccination. In contrast, IgE-specific antibody to F1 allergen rose significantly in a considerable number of the vaccinees. The results obtained indicate possible contamination of vaccine products with allergens of egg origin and a potential risk of allergic manifestation after influenza vaccination.

### INTRODUCTION

At present in Japan, it is recommended that influenza virus vaccine is given annually to schoolchildren from kindergarten to senior high-school age on the theoretical basis that mass immunization to children, who play a central role in the dissemination of influenza, may diminish the intensity of epidemics in the general population (Francis, 1967). Although more efficient methods of virus purification and disruption have led to more effective and less-toxic vaccines, some reactogenicity still remains. Because of the fear that the morbidity associated with the vaccine may be greater than that due to influenza, the public has generally been reluctant to accept vaccination against influenza, especially for healthy children. It is therefore necessary to estimate the current vaccine products from the aspect of both their efficacy and adverse reactions.

It is well known that one of the contraindications for influenza vaccination is known allergy to egg protein or chicken feathers. However in practice, only a brief history is taken from children or their parents in order to exclude those known to be allergic to these two allergens. Several serological tests, such as that for IgE-specific antibody to an allergen, are now available to make a definitive diagnosis of relevant allergies. The study described in this communication was initiated to estimate the frequency of individuals allergic to egg protein on the basis of current serological tests. Further the possibility that vaccinees would respond serologically to egg protein contaminating vaccines produced in eggs was investigated.

*Vaccine*

## MATERIALS AND METHODS

The vaccine employed in the study was an ether split-product vaccine commercially prepared by Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan. It contained 200 chick cell agglutinating (CCA) units per ml of A/Bangkok/10/83 (H1N1), 350 CCA units per ml of A/Philippines/2/82 (H3N2), and 150 CCA units per ml of B/USSR/100/83. The nitrogen contents in the respective preparations were determined to be 7, 15, and 6  $\mu\text{g/ml}$ . Doses (0.5 ml) of the vaccine were given subcutaneously twice with a 3-week interval in the winter of 1985.

*Vaccinees*

A total of 100 vaccinees were selected from the students of Kumamoto National Nursing School and Fukuoka East National Nursing School. Before immunization, physicians confirmed that there was no history of allergy to egg or chicken feathers, acute or chronic illness and that none were pregnant. Almost all the students had received influenza vaccination annually for the last 3 years. The sera were collected before immunization and 3 weeks after the second doses.

*Serological tests*

(1) The standard haemagglutination-inhibition (HI) test was performed using the vaccine strains, A/Bangkok/10/83(H1N1), A/Philippines/2/82(H3N2) and B/USSR/100/83, as previously described (Yamane *et al.* 1981).

(2) The concentrations of total IgE and IgE-specific antibody to egg white (F1) allergen were determined by use of commercial test kits (Phadezym IgE PRIST and Phadezym RAST, Pharmacia Diagnostics, Piscataway, NJ, USA, respectively). The concentration of IgE-specific antibody to *Dermatophagoides pteronyssinus* (DP) was determined as a control.

(3) The IgG-specific antibodies to ovalbumin (OA) and ovomucoid (OM) antigens were determined by enzyme-linked immunosorbent assay, as previously reported (Hosoi *et al.* 1985; Yamane & Uemura, 1986). The OA and OM antigens were chromatographically purified from the commercial products (Sigma Chemical Co., St Louis, MO, USA) prior to use. Briefly, 200  $\mu\text{l}$  of serum sample diluted 210-fold with phosphate-buffered saline containing 0.1% human serum albumin were first applied onto a polyethylene bead coated with OA or OM antigens. After 3 h incubation at room temperature, the allergen beads were washed three times with 0.85% NaCl containing 0.04% Tween 20. A 200  $\mu\text{l}$  aliquot of anti-human IgG monoclonal antibody conjugated with horseradish peroxidase was added to the beads, and the beads were incubated overnight (16–20 h) at room temperature. After washing three times, the reagent mixtures of  $\text{H}_2\text{O}_2$  and 2,2'-azino-di-(3-ethyl benzthiazoline-6-sulphonic acid) were added as substrate, and then incubated for 30 min at room temperature. The enzymatic reaction was stopped by addition of  $\text{NaN}_3$  to give a final concentration of 0.003%. The colour developed in the reaction mixture was quantitated as the optical density at 415 nm. The concentration of IgG-specific antibody to OA or OM antigens is given in IgG reference units per ml by comparison with a positive reference sera (Yamane & Uemura, 1986).

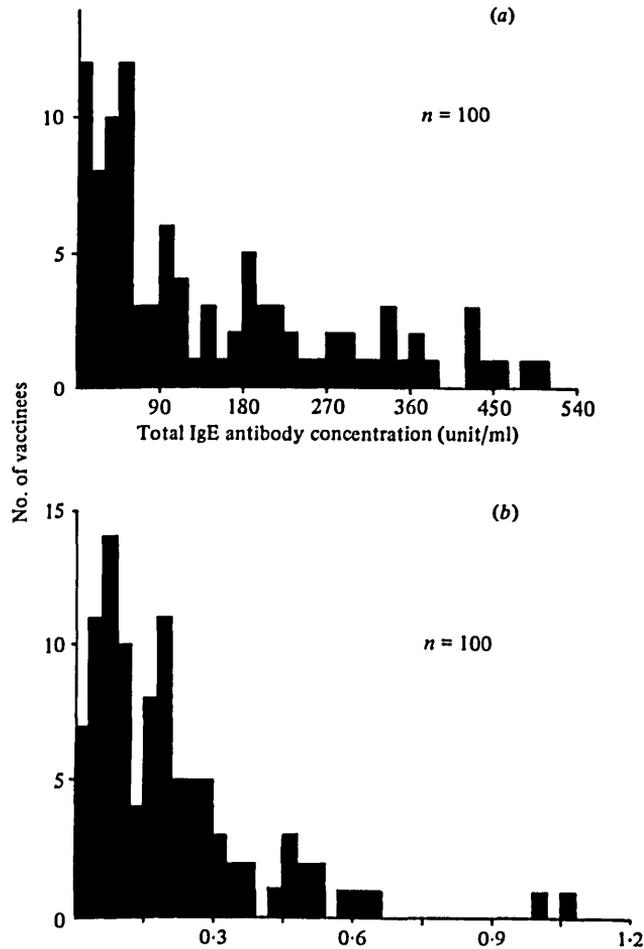


Fig. 1. The distributions of (a) total IgE and (b) IgE-specific antibody to egg white (F1) allergen before immunization.

### Statistical analysis

The geometric mean titres of HI antibodies and the geometric mean concentrations of IgE and IgG antibodies were calculated with log transformation, and Student's paired *t* test was performed as previously described (Yamane *et al.* 1981).

## RESULTS

### *The distributions of total IgE and IgE-specific antibody to F1 allergen before immunization*

Fig. 1 shows the distributions of total IgE concentrations and of IgE-specific antibody to F1 allergen among vaccinees before immunization. Both distributions represent log-normal distribution profiles. The geometric mean concentrations were calculated to be 77.6 units/ml (20.9–288.4 units/ml; mean  $\pm$  standard deviation) and 0.145 Phadezym RAST units (PRU)/ml (0.06–0.35 PRU/ml; mean  $\pm$  standard deviation), respectively. The concentrations of total IgE ranged

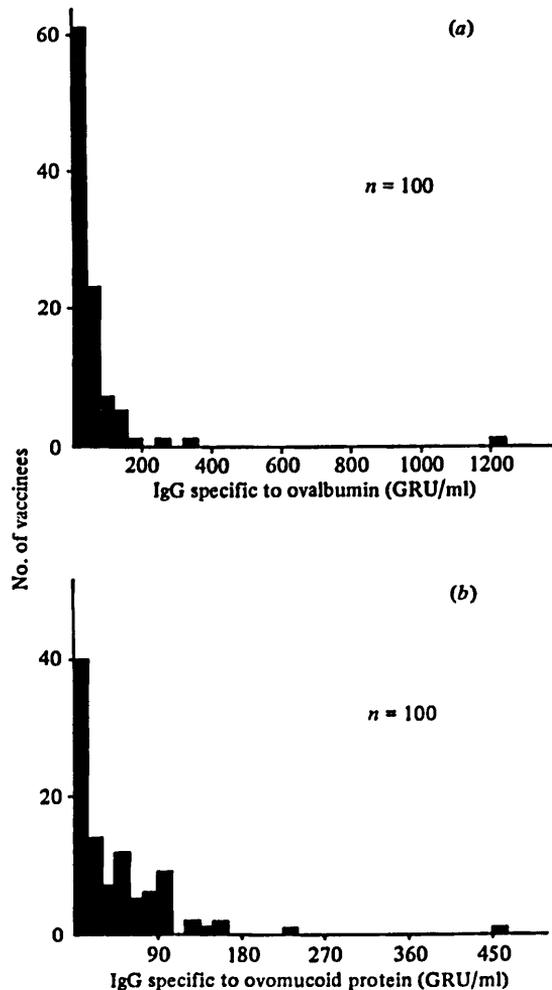


Fig. 2. The distributions of IgG-specific antibodies to the egg proteins, (a) ovalbumin (OA) and (b) ovomucoid protein (OM), before immunization.

from 1.5 to 500 units/ml, and those of IgE-specific antibody to F1 were from 0.02 to 1.06 PRU/ml. In our laboratory, concentrations of total IgE greater than 360 units/ml and those more than 0.35 PRU/ml for specific IgE are regarded as significantly high. Consequently, it was estimated that 10 vaccinees (10.0%) had high total IgE concentrations and 16 (16.0%) had high IgE-specific antibody to F1 allergen before immunization.

*The distribution of IgG-specific antibody to egg protein before immunization*

Fig. 2 shows the distributions of IgG-specific antibodies to egg protein, OA (Fig. 2a) and OM (Fig. 2b) antigens. Most vaccinees showed negligibly low concentrations of IgG antibodies to OA and OM antigens. However, assuming that the concentrations more than 100 IgG reference units (GRU)/ml are significant, the frequencies of vaccinees whose sera contained higher IgG antibody concentrations to OA and OM antigens were 12.0 and 10.0%, respectively.

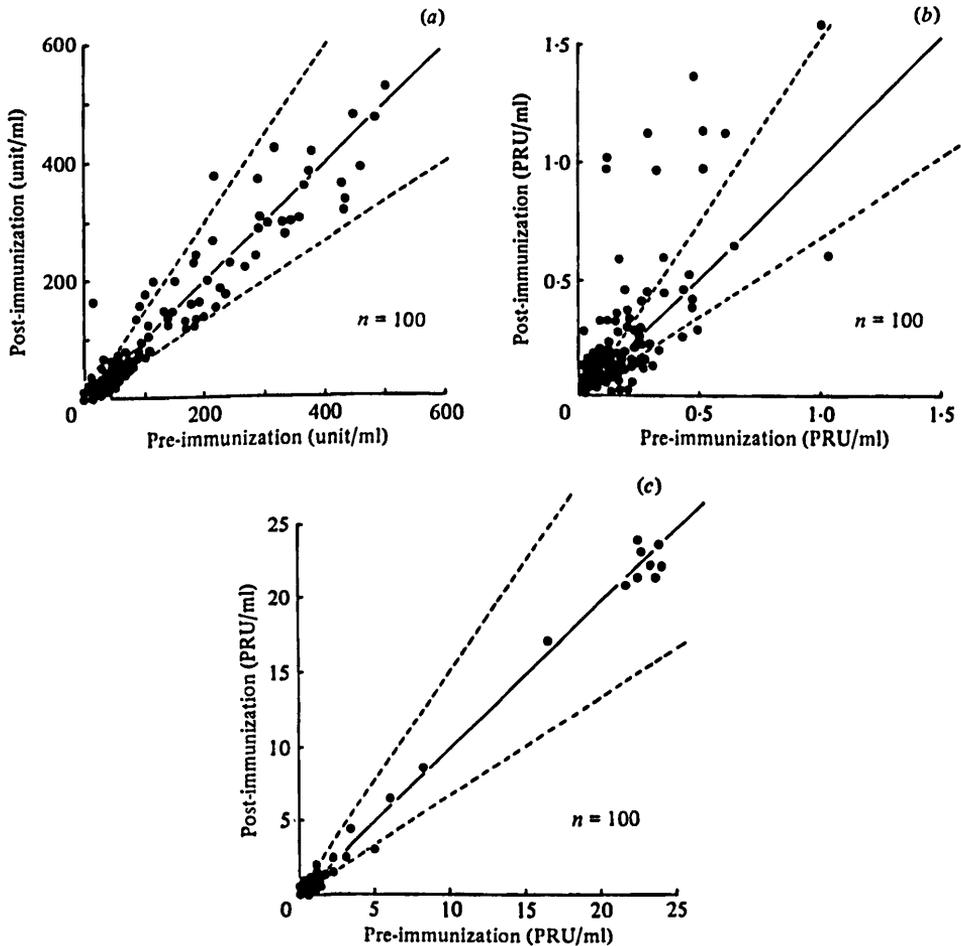


Fig. 3. Comparisons of (a) total IgE, (b) IgE-specific antibody to egg white (F1) and (c) IgE-specific antibody to *Dermatophagoides pteronyssinus* (DP) before and after immunization. The broken line indicates a 1.5-fold antibody change.

No significant correlations between past vaccination history and IgE and IgG antibody titres were demonstrated. Also, the concentrations of total IgE, IgE specific for F1, and IgG specific to OA and OM antigens in the sera taken before immunization were independent of each other. The correlation coefficients ranged from 0.003 to 0.409.

#### *The responses of total IgE and IgE specific to F1 allergen after immunization*

Fig. 3 shows the changes in total IgE and IgE antibody to F1 between pre- and post-immunizations. In most vaccinees, the changes in total IgE were not significant. None of the vaccinees whose sera contained higher titres (more than 360 units/ml) of total IgE before immunization showed a significant rise (1.5-fold or greater). The regression line between pre- and post-immunization was calculated to be  $y = 0.95x + 5.1$  (correlation coefficient; 0.946). On the other hand, significant rises in IgE specific to F1 after immunization were demonstrated in a considerable number of vaccinees. Of 16 vaccinees whose sera contained more than 0.35 PRU/

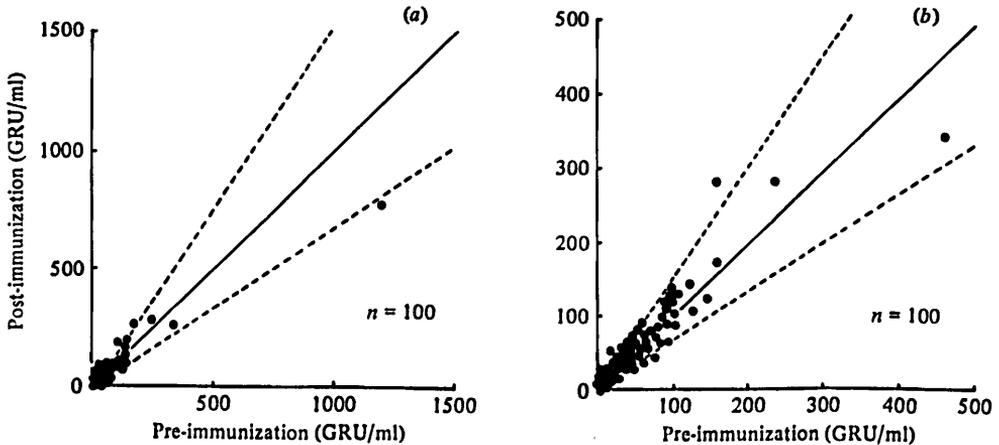


Fig. 4. Comparison of IgG-specific antibodies to the egg proteins, (a) ovalbumin (OA) and (b) ovomucoid protein (OM), before and after immunization. The broken line indicates a 1.5-fold antibody change.

ml of IgE specific to F1, six paired sera (37.5%) showed significant rises (1.5-fold or greater). In total, 36 of the 100 vaccinees showed significant rises in IgE specific to F1 allergen. Contrary to these results, significant IgE-specific antibody responses to DP allergen were not demonstrated.

Twenty-six vaccinees were shown to possess higher titres of IgE specific to DP allergen (more than 0.35 PRU/ml) before immunization, but only two vaccinees (7.7%) showed 1.5-fold or greater rises after immunization (see Fig. 3c).

#### *The responses of IgG-specific antibody to egg protein after immunization*

Fig. 4 shows the changes in IgG-specific antibodies to OA and OM antigens between pre- and post-immunizations. In most cases, the changes were not significant. Of 12 vaccinees whose sera contained more than 100 GRU/ml of specific IgG to OA, and of 10 vaccinees with such concentrations to OM before immunization, two cases to OA and only one to OM were regarded as significant rises (1.5-fold or greater). The regression line between pre- and post-immunizations were calculated to be  $y = 0.68x + 17.8$  (correlation coefficient; 0.953) for IgG to OA, and to be  $y = 0.95x + 6.48$  (correlation coefficient; 0.943) to OM.

#### *The antibody responses to influenza haemagglutinin after immunization*

In Table 1, the results of geometric mean titres of HI antibodies to vaccine strains, A/Bangkok/10/83(H1N1), A/Philippines/2/82(H3N2) and B/USSR/100/83 are summarized. Although the HI titres before immunization in most vaccinees were relatively high, some statistically significant rises in geometric mean titres could be demonstrated. The numbers of vaccinees whose sera showed significant rises (fourfold or greater) between pre- and post-immunizations were 23 (23.0%) to A/Philippines/2/82(H3N2), but only one to A/Bangkok/10/83(H1N1) and only two to B/USSR/100/83. There was no correlation between the HI antibody responses and those of IgE (total or specific to F1) or IgG specific to OA and OM antigens.

Table 1. Geometric mean titres (g.m.t.) of total IgE, IgE specific to allergens, IgG specific to egg proteins, and HI antibodies before and after immunization

Test	G.m.t. of sera collected		% with significantly high titres*	
	Pre-immunization	Post-immunization	Pre-immunization	Post-immunization
Total IgE (PRIST)	78.5	75.6	10.0	11.0
IgE specific to F1 (RAST)	0.145	0.152	16.0	26.0
IgE specific to DP (RAST)	0.196	0.205	26.0	27.0
IgG specific to OA	30.0	33.8	12.0	9.0
IgG specific to OM	16.1	21.4	10.0	13.0
HI antibody titres to	G.m.t. of sera collected		% with significantly antibody rises† after immunization	
	Pre-immunization	Post-immunization		
A/Bangkok/10/83 (H1N1)	522.7	652.5	1.0	
A/Philippines/2/82 (H3N2)	184.8	372.2	23.0	
B/USSR/100/83	125.4	171.2	2.0	

\* The titres of 360 units/ml or more in PRIST, of 0.35 PRU/ml or more in RAST, and of 100 GRU/ml or more in IgG specific to egg protein, were regarded as significantly high.

† Significant antibody rises were estimated as fourfold or greater rises in HI titres.

## DISCUSSION

The most troublesome adverse reactions associated with influenza vaccination are allergic manifestations, such as anaphylactic shock, bronchial asthma, or urticaria. Although the reported frequencies of allergic reactions were negligibly low in Japan, that is approximately 1/25000 (Tsunoda *et al.* 1980), it was also noticed that allergic reactions were observed with higher frequencies among children having egg allergy. The accumulated results indicated that the occurrence of deterioration of bronchial asthma was 2.1%, and that of systemic urticaria was 0.4% (Shioda *et al.* 1973). At present in Japan, over 15000000 schoolchildren annually receive influenza vaccination according to the national programme. This study was initiated to estimate a potential risk of allergic reactions during influenza vaccination by the serological tests for allergy.

In this study, the vaccinees were first screened by routine history taking to eliminate those with known allergy, especially to egg proteins or chicken feathers. However, it became apparent that a considerable number of vaccinees possessed significantly high titres of total IgE antibody and of IgE specific to egg white (F1) allergen. The frequencies were found to be 10–16%, respectively. These values were unexpectedly high when we assumed that the persons having allergic diathesis were excluded from the study. It is well known that most reaginic activity in human sera is due to IgE antibody (Ishizaka & Ishizaka, 1967; Ishizaka & Ishizaka, 1968). Davies *et al.* (1976) reported the correlation between IgE antibody to F1 and skin test using influenza vaccination products. The results

indicated that 7 of 22 vaccinees who were confirmed to be allergic against egg by specific IgE antibody testing were also positive in the skin test, and that two vaccinees gave a history of allergic adverse reactions in the past to influenza immunizations. Also, we observed febrile reactions to two vaccinees who received a cold-adapted reassortant live influenza virus vaccine, one of whom was positive in a skin test to egg allantoic fluid (Yamane *et al.* 1984). Although the number of vaccinees involved in the study was limited, and no significant adverse reactions became apparent during the study period, the results obtained suggest that there is a risk of allergic reaction among the vaccinees.

The role of IgG antibody in allergic reactions is not well investigated. However, there have been reports that IgG fractions also have reaginic activity as short-term sensitizing antibody to mast cells existing in lung and skin tissue (Parish, 1970). Our study revealed that several vaccinees possessed higher concentrations of IgG-specific antibodies to egg protein, OA and OM antigens. It is likely that these IgG antibodies also represent a potential risk of allergic adverse reactions, especially after subcutaneous injections.

In most vaccinees, IgG-specific antibody to egg protein did not rise significantly after immunization. It is thus likely that the current vaccine product does not contain enough egg protein to stimulate an IgG antibody response. Contrary to the IgG response, IgE specific to F1 rose significantly after immunization in a considerable number of vaccinees, the results suggesting that influenza vaccine may play a role in sensitizing an individual to egg protein. The contamination of influenza virus and Japanese encephalitis virus vaccines by egg protein has been reported following the observation that IgG-specific antibody to ovomacroglobulin became detectable in rabbits after immunizations (Kawashima, 1986). Also, Tsuji *et al.* (1983) suggested that the relatively higher titres of specific antibodies (IgG and IgE) to egg protein among Japanese might be a consequence of annual immunization against influenza. Although the improved industrial processes may eliminate nearly all contamination from allantoic fluid, it will be necessary to establish an assay technique to detect trace amounts of contamination from eggs and to estimate, on a large scale, the effect of annual immunization on allergic manifestations.

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