

Short communication

Specific IgE and IgG to gelatin in children with systemic cutaneous reactions to Japanese encephalitis vaccines

Background: Systemic allergic reactions to Japanese encephalitis (JE) vaccine that include urticaria, angioedema, and rash have been reported. In Japan, children who suffered from allergic immediate-type reactions to JE vaccine had antigelatin IgE in their sera. However, the immunologic mechanism of allergic nonimmediate-type reactions that consist of cutaneous signs appearing several hours or more after JE vaccination has not been defined.

Methods: Serum samples were taken from 28 children who showed allergic nonimmediate-type cutaneous reactions to JE vaccine. Furthermore, serum samples were taken from 10 children who showed allergic immediate-type reactions with cutaneous signs and/or respiratory symptoms to JE vaccine. We have defined an immediate-type reaction as one occurring within 1 h after vaccination.

Results: Of 10 children who showed immediate-type reactions, all had antigelatin IgE and IgG. Of 28 children who showed systemic nonimmediate-type reactions, one had antigelatin IgE and nine (32%) had antigelatin IgG. The child who had antigelatin IgE showed urticaria 2 h after JE vaccination.

Conclusions: These results suggest that some children who showed allergic nonimmediate-type reactions to JE vaccine were sensitized to gelatin.

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Systemic allergic reactions to Japanese encephalitis (JE) vaccine that include urticaria, angioedema, and rash have been reported (1–7). JE vaccine has been administered to children in Japan. We found that most children who had allergic immediate-type reactions to live virus vaccines with gelatin had antigelatin IgE (8–11). All inactivated JE vaccines in Japan also contain gelatin as a stabilizer (12). Recently, we found that children who suffered from allergic immediate-type reactions (cutaneous and/or respiratory symptoms) to JE vaccine had antigelatin IgE in their sera (13).

In this study, we observed that children showed allergic nonimmediate-type reactions that consisted of systemic cutaneous signs appearing several hours or more after JE vaccination. The immunologic mechanism of the nonimmediate-type reactions to JE vaccines has not been defined.

Recently, it was found that some of the children who had similar nonimmediate-type reactions to live virus vaccines but had no antigelatin IgE showed gelatin-specific lymphocyte proliferation and the presence of antigelatin IgG (14–16). These results suggest that immune responses to gelatin may have some role in the pathogenesis of nonimmediate reactions to these live virus vaccines with gelatin.

To investigate the relationship between immune-responses to gelatin and allergic nonimmediate-type

reactions to JE vaccine, we measured gelatin-specific IgE and IgG in the sera of children who had the allergic reactions.

Material and methods

Children

From May 1995 to January 1998, we received from physicians and vaccine manufacturers serum samples taken from children who had experienced allergic immediate-type and nonimmediate-type reactions to JE vaccine. We defined an immediate-type reaction as an IgE-mediated reaction occurring within 1 h after vaccination.

We studied 10 children (two male, eight female; mean age $\pm$ SD, 4 years $\pm$ 7 months) who experienced systemic immediate-type reactions to JE vaccine. Of these 10 children, four suffered from mild anaphylaxis: systemic cutaneous signs (e.g., urticaria, angioedema) and respiratory symptoms (wheezing, cough, laryngeal edema) without severe symptoms (airway obstruction, anaphylactic shock); six displayed only allergic cutaneous signs. None suffered severe anaphylaxis; that is, cutaneous signs plus airway obstruction or anaphylactic shock. These systemic reactions appeared within 1 h after JE vaccination (mean time $\pm$ SD, 33 $\pm$ 22 min).

We also studied 28 children (14 male, 14 female) (mean age $\pm$ SD, 4 years 2 months $\pm$ 11 months) who had allergic nonimmediate-type reactions to JE vaccine. Of 28 children, 14 had only urticaria, six urticaria and angioedema, and eight erythema multiforme or nodosum. Their reactions appeared 2–48 h after JE vaccination (mean time $\pm$ SD, 19 $\pm$ 17 h).

As a negative control, 15 children (seven male, eight female, mean age $\pm$ SD, 5 years $\pm$ 1 year 1 month) had no allergic reaction to JE vaccine containing gelatin.

JE vaccines

In Japan, all of the inactivated and purified JE vaccines, which are derived from infected mouse brain, contain gelatin (0.05–0.1 mg/dose) as a stabilizer.

Measurement of gelatin-specific IgE antibody

The CAP System (Pharmacia, Uppsala, Sweden) was used to determine the concentration (Ua/ml) of IgE to bovine gelatin (Wako Pure Chemical Industries, Osaka, Japan) (13). This method was performed according to the manufacturer's instructions (17).

Measurement of gelatin-specific IgG antibody

Anti-bovine-gelatin IgG levels were measured by ELISA (15). Microplates were coated with bovine gelatin (1 μ g/ml) in the presence of bovine serum albumin (BSA) (30 μ g/ml) overnight at 4°C. Each patient's serum, diluted 1:100 and 1:1000 with 20% fetal calf serum in phosphate-buffered saline with 0.05% Tween 20, was incubated in microplate wells at room temperature for 90 min. After washing, antihuman IgG conjugated with peroxidase (Biosource International, Camarillo, CA, USA) was added to each well. Color was then developed with a mixture of hydrogen peroxide and *o*-phenylenediamine dihydrochloride. After the enzyme reaction was stopped with 4 N sulfuric acid, the absorbance at 492 nm was measured with a colorimetric microplate reader (Flow Laboratories, McLean, VA, USA). The level of specific antibodies was represented as an arbitrary unit (U)/ml. The U/ml of each serum was calculated from standard titration curves of a pooled serum that showed high levels of antigelatin IgG. The cutoff value of antigelatin IgG has been described previously (15). The specificity of the ELISA was evaluated by inhibition test by addition of fluid-phase gelatin and BSA. Gelatin (100 μ g/ml) completely inhibited antigelatin IgG, but BSA (100 μ g/ml) did not (data not shown).

Results

Antigelatin IgE levels in children with allergic immediate- and nonimmediate-type reactions to JE vaccines

Fig. 1 shows the relationship between antigelatin IgE levels in the sera of affected children and the time of onset of allergic reactions after JE vaccination. All 10 children who showed immediate-type reactions had antigelatin IgE ranging from 1.1 to 29.5 Ua/ml (mean value $\pm$ SD, 12.2 ± 7.8 Ua/ml) (Fig. 1). There was no significant difference in antigelatin IgE levels between children with anaphylaxis and those with only cutaneous signs. We confirmed that there was a strong relationship between the immediate-type reactions to JE vaccines and the presence of antigelatin IgE in these children's sera.

Of 28 children who showed allergic nonimmediate-type reactions to JE vaccine, one had antigelatin IgE (Fig. 1). This child showed urticaria 2 h after the JE vaccination. The other children had no antigelatin IgE.

In a negative control experience, all 15 children who had no reactions to JE vaccine had no antigelatin IgE.

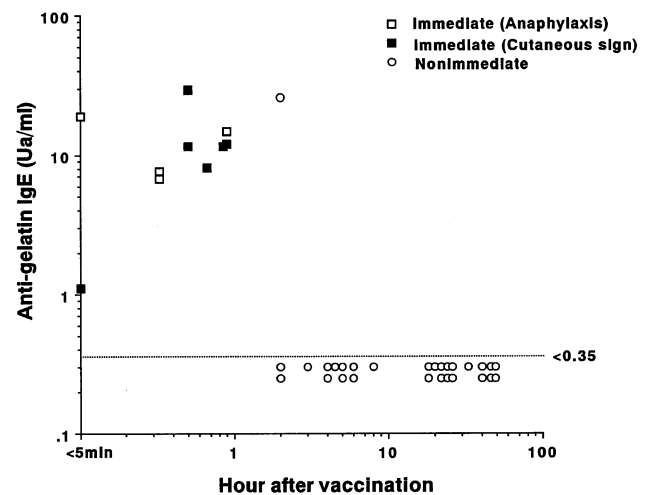


Figure 1. Relationship between antigelatin IgE level and time onset of allergic reactions after JE vaccination. Immediate-type reactions (including anaphylaxis) ($\square$), immediate-type reactions (only cutaneous signs) ($\blacksquare$), nonimmediate-type reactions ($\circ$).

Antigelatin IgG levels in children with allergic immediate- and nonimmediate-type reactions to JE vaccine

Fig. 2 shows the relationship between antigelatin IgG levels in the sera of affected children and the time of onset of allergic reactions after JE vaccination. All 10 children who showed systemic immediate-type reactions to JE vaccine had antigelatin IgG, ranging from 17 to 4560 U/ml (mean value $\pm$ SD, $1010 \pm 1,600$ U/ml) (Fig. 2). There was no significant difference in antigelatin IgG levels between children with anaphylaxis and those with only cutaneous signs.

Of 28 children who showed allergic nonimmediate-type reactions to JE vaccine, nine (32%) had antigelatin IgG ranging from 13 to 614 U/ml (mean value $\pm$ SD, 99 ± 196 U/ml) (Fig. 2). The child with antigelatin IgE also had antigelatin IgG.

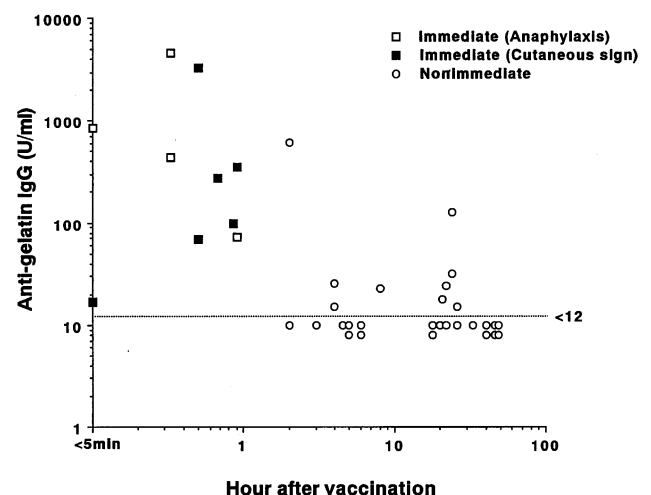


Figure 2. Relationship between antigelatin IgG level and time onset of allergic reactions after JE vaccination. Immediate-type reactions (including anaphylaxis) ($\square$), immediate-type reactions (only cutaneous signs) ($\blacksquare$), nonimmediate-type reactions ($\circ$).

In a negative control experiment, all 15 children who had no reactions to JE vaccine had no antigelatin IgG.

Discussion

All the children who had allergic immediate-type reactions with cutaneous signs were found to have anti-bovine-gelatin IgE. We found a strong relationship between allergic immediate-type reactions to JE vaccine and the presence of specific IgE to gelatin. This confirms the findings of our previous study (13). We also found a similar relationship between allergic immediate-type reactions to live virus vaccines (e.g., measles, rubella, mumps, varicella vaccines) containing gelatin and the presence of antigelatin IgE (8–11).

To investigate the relationship between the immune response to gelatin and nonimmediate-type reactions to JE vaccine, we measured antigelatin IgG levels in the sera of children who had experienced allergic reactions to JE vaccine. We found that some of the children who showed nonimmediate-type reactions had antigelatin IgG.

In allergic diseases, however, the role of IgG-dependent reactions has long been debated (18). Some investigators have reported that IgG antibodies can act as a sensitizing antibody (19–21). In guinea pig, IgG1 and IgG2 are the essential antibodies for passive anaphylactic shock (22). Basophils and mast cells, besides FcεRI, have FcεRII or FcεRIII that binds IgG (23, 24), and basophils or mast cells can be activated through FcεRIII to release histamine (25). Moreover, the cell-mediated hypersensitivity, which appears only after several hours, reached a maximum at 24–48 h (26).

Our previous study found that there is a relationship between antigelatin IgG and cell-mediated responses to gelatin; that is, children who had antigelatin IgG showed cell-mediated responses to gelatin (14). Similarly, immune complex-mediated (type III) hypersensitivity in the skin is characterized by erythema, and edema maximal at 3–8 h (Arthus reaction) (25). The specific IgG may form immune complexes with gelatin. However, it is unclear whether gelatin-specific IgG causes the systemic nonimmediate-type reactions discussed in this report. We think that it is worth noting that the children who had both nonimmediate-type reactions and antigelatin IgG might have been sensitized to gelatin.

In this study, one child who showed systemic urticaria 2 h after JE vaccination had antigelatin IgE and IgG. This JE vaccine contains only 0.1 mg gelatin per dose, and we elsewhere reported that more than 1.0 mg of gelatin in measles, mumps, and rubella vaccines causes anaphylaxis (9). The symptoms with JE vaccination were milder than those with measles and mumps vaccines, which contain more than 1 mg of gelatin per dose (13). It is possible that allergic reactions in the child would appear late owing to a smaller amount of gelatin in the JE vaccine.

At present, the mechanism and cause of systemic nonimmediate-type reactions to JE vaccines have not been clearly established. This study suggests that some children who showed nonimmediate-type reactions had been sensitized to gelatin.

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