Jpn. J. Infect. Dis., 53, 189-195, 2000

Review

Systemic Allergic Reactions to Gelatin Included in Vaccines as a Stabilizer

Masahiro Sakaguchi* and Sakae Inouye[1]

Department of Immunology and
[1]Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan

(Received September 28, 2000. Accepted November 7, 2000)

CONTENTS:

SUMMARY: Most of the children who showed systemic immediate-type reactions, including anaphylactic shock, to measles, mumps, rubella, and varicella vaccines had IgE antibodies to gelatin; thus we suspected that the allergic symptoms are caused by gelatin antigen, which is usually included in these live-virus vaccines as a stabilizer. We hypothesized that the anti-gelatin IgE is elicited by immunization with DTaP (diphtheria-tetanus-acellular pertussis) vaccines, which contained a small amount of gelatin as a spillover protein after purification of pertussis toxin. To test this hypothesis, we conducted a case-control study to determine whether children with anti-gelatin IgE had received gelatin-containing DTaP vaccines, and it was indeed found that all such children in the study had immunization histories that included the gelatin-containing DTaP vaccines. Based on these findings, the vaccine manufacturers had removed gelatin from all the DTaP and live-virus vaccines produced in Japan by 2000.

1. Systemic immediate-type reactions to gelatin in live-virus vaccines

1.1 Introduction

Reports of anaphylaxis in response to measles-mumps-rubella (MMR) vaccines have been extremely rare (1,2). Although more than 70 million doses of MMR vaccines were used in the United States (US) from 1990-1995, only 33 cases of anaphylactic reactions were reported (3,4). These adverse reactions were also thought to be rare in Japan before 1993, when a Japanese case of anaphylaxis in response to measles was first reported (5). Since then, many children have been reported as showing systemic immediate-type reactions, including anaphylactic shock to measles, mumps, rubella, and varicella vaccines in Japan (6-9). It has been suggested that anaphylaxis to MMR vaccines that are derived from chick embryo cells is caused by allergy to egg proteins present in the vaccines (10). However, these systemic immediate-type reactions have also been described in children who tolerated eggs (11-13). It has therefore been suggested that anaphylaxis-inducing components other than egg protein may be present in these vaccines (2).

The anaphylactic reactions to these vaccines are thought to be caused by gelatin allergy: Kelso et al. detected anti-gelatin IgE in a child who suffered from anaphylaxis in response to gelatin-containing MMR vaccine (3). We have also detected anti-gelatin IgE in many children with systemic immediate-type reactions to measles, mumps, and rubella vaccines (6,7). Further, we have detected anti-gelatin IgE in children showing systemic immediate-type reactions to varicella and Japanese encephalitis virus vaccines (14,15). We suspected that most of the systemic immediate-type reactions occurring after vaccination were caused by the gelatin present in the vaccines as a stabilizer. However, the mechanism of sensitization to gelatin has remained uncertain (16).

1.2 Systemic immediate-type reactions to gelatin in live-virus vaccines

Systemic immediate-type reactions generally appear within 1 h of vaccination (17). Systemic immediate-type reactions are classified into three groups: a) severe anaphylaxis consisting of cutaneous signs (e.g., systemic urticaria, angioedema) plus airway obstruction (with laryngeal edema or wheezing) or anaphylactic shock (with hypotension and vascular collapse); b) mild anaphylaxis consisting of cutaneous signs plus respiratory symptoms (wheezing and/or cough or laryngeal edema) without severe symptoms (e.g., airway obstruction); and c) systemic cutaneous signs consisting only of systemic urticaria or angioedema.

Measles vaccines are produced by four manufacturers in

*Corresponding author: Tel: +81-3-5285-1111, Fax : +81-3-5285-1156, E-mail: msakaguc@nih.go.jp

Japan, and all the vaccines previously contained bovine gelatin in amounts ranging from 1 to 10 mg per dose. Rubella vaccines are produced by five manufacturers, three of which produced rubella vaccines containing bovine gelatin in amounts ranging from 1 to 2.5 mg per dose. Mumps vaccines are produced by four manufacturers, two of which produced mumps vaccines containing bovine gelatin in amounts ranging from 1 to 10 mg per dose (17).

In our summary data (17), we found a strong relationship between the immediate-type reactions after measles, mumps, and rubella vaccinations and the presence of anti-gelatin IgE in sera of the children studied. Table 1 shows data from 111 children who had systemic immediate-type reactions to measles vaccines between 1994 and 1996. Of the 111 children, 24 showed severe anaphylaxis, 35 had mild anaphylaxis without airway obstruction or shock, and 52 had systemic cutaneous signs only. Of the 111, 94 children (86%) had anti-gelatin IgE (17). Table 2 shows the data from 50 children who had systemic immediate-type reactions to rubella vaccines. In 1994, we found no cases of systemic immediate-type reactions to this vaccine, probably because few infants received the vaccine before 1994. Of the 50 children, 11 showed severe anaphylaxis, 13 mild anaphylaxis, and 26 only generalized urticaria. Of the 50, 46 children (92%) had anti-gelatin IgE. Table 3 shows data from 17 children who had systemic immediate-type reactions to mumps vaccines. Of the 17 children, 2 showed severe anaphylaxis, 7 mild anaphylaxis, and 8 only urticaria. All 17 children had anti-gelatin IgE. We therefore concluded that most of the systemic immediate-type reactions occurring after vaccination are caused by the gelatin present in the vaccines as a stabilizer.

Table 1. Systemic immediate-type reactions to measles vaccines in children

Year[a]	Anaphylaxis		Cutaneous signs	Total
	Severe	Mild		
1994	5(5)[b]	4(3)	9(7)	18(15)
1995	10(9)	14(13)	17(16)	41(38)
1996	9(7)	17(17)	26(17)	52(41)
Total	24(21)	35(33)	52(40)	111(94)

[a] Japanese statistical year; from April to next March.
[b] Number in parentheses is the number of children with anti-gelatin IgE.

Table 2. Systemic immediate-type reactions to rubella vaccines in children

Year[a]	Anaphylaxis		Cutaneous signs	Total
	Severe	Mild		
1994	0	0	0	0
1995	6(6)[b]	3(3)	10(9)	19(18)
1996	5(5)	10(10)	16(13)	31(28)
Total	11(11)	13(13)	26(22)	50(46)

[a,b] The same as in Table 1.

Table 3. Systemic immediate-type reactions to mumps vaccines in children

Year[a]	Anaphylaxis		Cutaneous signs	Total
	Severe	Mild		
1994	0	3(3)[b]	0	3(3)
1995	1(1)	2(2)	4(4)	7(7)
1996	1(1)	2(2)	4(4)	7(7)
Total	2(2)	7(7)	8(8)	17(17)

[a,b] The same as in Table 1.

In Japan, a varicella vaccine containing gelatin has been licensed for normal and high-risk children since 1986 (18). This vaccine has protected children against varicella infection, and its safety has been confirmed in a clinical trial (18). The live varicella vaccine is derived from infected human embryo lung cells and contains both bovine gelatin (1 mg/dose) and hydrolyzed gelatin (12.5 mg/dose) as stabilizers.

In our 1997 study (14), we found that children inoculated with the varicella vaccine exhibited systemic allergic reactions with the synthesis of anti-gelatin IgE. Table 4 shows data from 30 children with systemic immediate-type reactions to varicella vaccines. Of the 30 children, 7 showed severe anaphylaxis, 9 mild anaphylaxis, and 14 only urticaria (19). Of these 30, 27 children (90%) had anti-gelatin IgE. Since this vaccine is derived from human embryo cells, we can rule out the possibility of an etiologic relationship between egg proteins and the anaphylactic reactions. These findings suggest that such reactions are caused by the gelatin contained in the vaccine. The gelatin-containing varicella vaccine must therefore be used with the same caution as the MMR vaccine.

In response to these results, the gelatin in all measles, mumps, rubella, and varicella vaccines produced in Japan was eliminated or replaced with gelatin of low allergenicity between 1997 and 2000 (personal communication from the manufacturers).

1.3 Estimate of the minimum incidence of anaphylaxis to gelatin in live-virus vaccines

Table 5 shows an estimate of the minimum incidence in the 1996 statistical year of severe anaphylaxis to the live-virus vaccines (17). The 1996 incidences of severe anaphylaxis to each gelatin-containing vaccine were 8.13, 7.80, 8.33, and 18.8 cases per million doses of measles, rubella, mumps, and varicella vaccines, respectively. The incidence of anaphylaxis to MMR vaccines in the US from 1990-1995 was reported as 0.5 cases per million doses (4), one order of magnitude less than that in Japan, suggesting that Japanese children were sensitized with anti-gelatin IgE at a higher rate than US

Table 4. Systemic immediate-type reactions to varicella vaccines in children

Year[a]	Anaphylaxis		Cutaneous signs	Total
	Severe	Mild		
1994	1(1)[b]	0	0	1(1)
1995	1(1)	2(2)	2(2)	5(5)
1996	5(5)	7(7)	12(9)	24(21)
Total	7(7)	9(9)	14(11)	30(27)

[a,b] The same as in Table 1.

Table 5. Estimate of the minimum incidence of severe anaphylaxis to four gelatin-containing vaccines

Severe anaphylaxis	Vaccine			
	Measles	Rubella	Mumps	Varicella
1994	4.49[a]	–[b]	0	5.26
1995	7.77	6.96	7.67	4.47
1996	8.13	7.80	8.33	18.8
Total	6.84	7.31	4.36	10.3

[a] The number of cases per million doses of each vaccine.
[b] Before 1994, rubella vaccine was used only for immunization of 15-year-old girls in Japan.

children. Nakayama et al. also reported the incidence of anaphylaxis to gelatin-containing vaccines from the Kitasato Institute (a vaccine manufacturer, Tokyo): 11.9 cases/million doses for the measles vaccine, 6.52 cases/million doses for the rubella vaccine, and 18.5 cases/million doses for the mumps vaccine (9).

1.4 Relationship between anti-gelatin IgE production and DTaP vaccination history

As described above, we suspected that most cases of anaphylaxis to live attenuated viral vaccines containing gelatin were caused by the gelatin. However, the mechanism of sensitization to gelatin remained uncertain. It has been revealed that the diphtheria-tetanus-acellular pertussis (DTaP) vaccines produced by some manufacturers contained a small amount of gelatin as a spillover protein during the process of purifying pertussis toxin (19). This suggested the possibility that anti-gelatin IgE may be produced by the gelatin-containing DTaP vaccines prior to immunization with gelatin-containing measles vaccines (9). Recently, Nakayama et al. reported that use of the gelatin-containing DTaP vaccine might be causally related to the development of gelatin allergy (9). To further evaluate this possibility, we compared the DTaP vaccination histories of two groups of children: a) children who had showed both anti-gelatin IgE and immediate-type reactions to measles, mumps, and rubella vaccines; and b) children who showed no reactions to these vaccines. All IgE-positive 54 (100%) children had received gelatin-containing DTaP vaccines (Table 6)(20). By contrast, of the 101 children used as a control, 72 (71%) had received DTaP vaccines with gelatin and 29 (29%) had received DTaP vaccines free of gelatin. Therefore, a highly significant relationship was found to exist between prior vaccination with gelatin-containing DTaP vaccine and subsequent anti-gelatin IgE production ($P < 0.001$) (Table 6) (20).

We suspect that the sudden increase in the incidence of anaphylaxis in the early 1990s is related to changes in the immunization schedule of DTaP vaccines (Fig. 1). In Japan, killed whole-cell pertussis vaccines were introduced for routine childhood vaccination at the end of the 1940s, followed by the introduction of combined whole-cell DTP (DTwP) vaccines (21). However, the report of two fatal incidents after DTwP vaccination in December 1974 and January 1975 led to the temporary suspension of DTwP vaccination. In April 1975, these vaccinations were resumed, with a change in the age of the initial routine immunization from 3 months to 2 years of age. In 1981, acellular pertussis vaccines replaced whole-cell vaccines for the immunization of 2-year-olds (21). In December 1988, following 7 years of safe and effective use of DTaP vaccines in older children, the Ministry of Health and Welfare recommended the use of DTaP vaccine for routine vaccination of 3-month-old infants (22). Since 1989, children 3 to 24 months old have been receiving DTaP vaccines. In 1994, the Preventive Vaccination Law was revised and all infants began to receive DTaP vaccines before measles vaccinations (23). Thus, the gelatin-containing DTaP vaccination administered at younger ages may have sensitized some children to gelatin. We assume that there is a relation between the change in the immunization schedule for DTaP vaccines and the emergence of anaphylaxis to the vaccines since 1993. Based on these findings, gelatin has been eliminated from all DTaP vaccines produced in Japan since 1998 (personal communication from the manufacturers).

Table 6. DTaP vaccination histories of children with anti-gelatin IgE and systemic immediate-type allergic reactions to vaccines

History of DTaP vaccine	Allergic reactions	
	+	−
with gelatin	54	72
without gelatin	0	29

Significant relationship between histories of vaccination with gelatin-containing DTaP vaccine and anti-gelatin IgE production ($P < 0.001$).

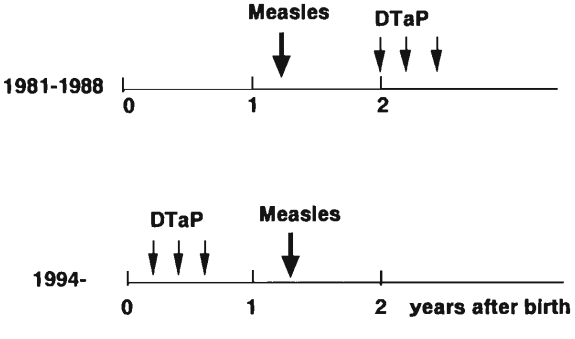

Fig. 1. Childhood immunization schedule in Japan.

2. Systemic nonimmediate-type reactions to gelatin in live-virus vaccines

We have observed systemic nonimmediate-type reactions, consisting of systemic cutaneous signs, appearing several hours or more after live-virus vaccination (24). Most of the patients demonstrating these nonimmediate-type reactions had no anti-gelatin IgE.

Kumagai et al. reported on cellular immune responses in children with both immediate- and nonimmediate-type reactions to vaccines (8). In their study, all six patients with immediate-type reactions had IgE to gelatin, whereas none of the 21 patients with nonimmediate-type reactions had IgE to gelatin. All six patients with immediate-type reactions and 17 of the 21 patients with nonimmediate-type reactions showed T cell responses specific to gelatin. In contrast, the tests were negative in 14 control subjects, who had no adverse reactions to the vaccines.

Taniguchi et al. reported that 61 of the 76 patients with nonimmediate-type reactions showed T cell responses specific to gelatin (25). In contrast, none of 14 control subjects with no adverse reactions to the vaccines had T cell responses specific to gelatin. Further, they reported that activated memory T ($CD4^+CD25^+CD45RO^+$) cells were induced by co-culture with gelatin in patients with nonimmediate-type reactions.

Ohsaki et al. further analyzed T cell responses in patients with reactions to gelatin-containing vaccines (26). In their study, all eight patients with immediate-type reactions and the eight patients with nonimmediate-type reactions showed T cell responses specific to gelatin. Lymphocytes of patients with immediate-type reactions expressed not only IL-4 and IL-13 mRNA but also IFN- and IL-2 mRNA. On the other hand, lymphocytes of patients with nonimmediate-type reactions expressed these cytokines very weakly or sometimes not at all.

The results of these studies suggest that an immune response to gelatin may also play a role in the pathogenesis of nonimmediate-type reactions to gelatin-containing live-

virus vaccines.

Further, we examined anti-gelatin IgG in sera of children who suffered from systemic adverse reactions after measles, rubella, and mumps immunization with gelatin-containing live-virus vaccines (27). Of 75 children who had nonimmediate-type reactions with no anti-gelatin IgE, 22 (29%) had anti-gelatin IgG. The IgG positivity was well correlated with the lymphocyte proliferation assay positivity. In contrast, as a negative control, none of the children with no allergic reaction to live- virus vaccines had anti-gelatin IgG. Systemic nonimmediate-type allergic reactions, which mainly consist of systemic cutaneous signs, appearing several hours or more after exposure, have also been reported after varicella vaccination. We obtained similar results in our study: some children with systemic non-immediate-type allergic reactions had anti-gelatin IgG (28). These results also suggest the possibility that some nonimmediate-type reactions to the varicella vaccine are caused by immune reactions to gelatin.

3. Systemic immediate-type reactions to gelatin in inactivated vaccines

Systemic allergic reactions to the Japanese encephalitis (JE) vaccine, including systemic urticaria and/or angioedema, have been reported (29-32). Inactivated JE vaccines also contain gelatin as a stabilizer. We reported in 1997 that children had systemic immediate-type reactions to the JE vaccine (15). In a subsequent study (33), we identified two different patterns of systemic immediate-type reactions to the JE vaccine containing gelatin as a stabilizer. One group of affected children had cutaneous and respiratory symptoms (e.g., systemic urticaria and wheezing), and the other showed cardiovascular symptoms (e.g., hypotension and cyanosis) without cutaneous and respiratory symptoms. The children in the former group had anti-gelatin IgE in their sera, whereas those in the latter group did not. We believe that the two patterns may have been caused by different mechanisms or different allergens. The immunological mechanism of the adverse reactions in the latter group has not yet been determined.

Recently, an incidence of systemic urticaria beginning 10 min after the administration of DTaP vaccine was reported in a child (34). Children immunized with DTP, DT, or T vaccines have also been reported to have specific IgE to the toxoids (35-37). Accordingly, it has been suggested that there is a relationship between anti-toxoid IgE and the local side effects to these vaccines (38).

In a 1998-study (19), we found that eight children had systemic urticaria within 30 min of the administration of DTaP vaccines that contained gelatin. We measured the levels of specific IgE to gelatin and DTP toxoids in their sera, and found that none of the children had anti-gelatin IgE. Of the eight children, two had detectable levels of anti-toxoid IgE. Ten children who showed no allergic reaction to the DTaP vaccines were used as controls. Among them, four had detectable levels of anti-toxoid IgE. Based on these results, there was no clear relationship between the specific IgE to the vaccine's component proteins and systemic urticaria to DTaP vaccines (19).

All DTaP vaccines contain thimerosal (a mercury-containing organic compound), which has been commonly used as an injectable drug preservative. Hypersensitivity to thimerosal has been reported to be common (39). Okada et al. reported that a child with systemic urticaria in response to DTaP vaccines had an immediate-type positive skin test to thimerosal (34). However, there is currently no method for measuring anti-thimerosal IgE. In the future, such a method should be developed.

4. Systemic immediate-type reactions to gelatin-containing non-vaccine items

Recombinant human erythropoietin (EPO) is widely used for the safe and effective treatment of anemia in chronic dialysis patients. There has been one report of anaphylaxis associated with EPO, in which the patient had IgE antibody to recombinant EPO (40). As a stabilizer for the injectable EPO product, human serum albumin (HSA) was replaced with bovine gelatin between 1995 and 1996 in Japan.

We previously reported the case of a woman who experienced anaphylaxis in response to the gelatin stabilizer in an EPO product. She had undergone intravenous EPO therapy since 1990 and had demonstrated no allergic reaction to HSA-containing EPO products. In 1995, she received a new EPO product that contained bovine gelatin as a stabilizer. Severe anaphylaxis (hypotension and airway obstruction with cough) began immediately after intravenous injection of the product. She was found to have anti-bovine gelatin IgE (13.7 Ua/ml), but no anti-EPO IgE (41). These results suggest that anaphylaxis to EPO products is associated with hypersensitivity to the gelatin included in the products.

In Japan, approximately 100,000 dialysis patients received EPO products containing gelatin from 1995 to 1996. Interestingly, the above-mentioned case was the only reported case in which severe immediate-type reactions to gelatin-containing EPO products occurred. Thus, this would appear to be an extremely rare case of hypersensitivity to bovine gelatin in the adult Japanese population.

It was reported that a woman who experienced urticaria

Table 7. Children with food allergy to gelatin

Child No.	Food allergy to gelatin	
	Timing	Symptom
2	Post	Systemic urticaria, angioedema around eyes, vomiting
4	Post	Systemic urticaria
12	Post	Systemic urticaria
15	Post	Systemic urticaria, vomiting, wheezing, cough
16	Post	Systemic urticaria, airway obstruction with laryngeal edema
21	Pre	Systemic urticaria
24	Pre	Systemic urticaria, lip swelling

Post: Gelatin food allergy occurred after allergic reactions by vaccination; Pre: Gelatin food allergy occurred before allergic reactions by vaccination.

after chewing gelatin-containing fruit gums had IgE antibody to gelatin as detected by radioallergosorbent assay (42). Kelso et al. reported that a patient with anaphylaxis to the MMR vaccine also had allergic reactions after eating gelatin-containing food (3). These findings suggest that some children who had anaphylaxis in response to vaccines may have a food allergy to gelatin.

We previously detected the occurrence of food allergy to gelatin in 7 of 26 children who showed allergic reactions to live-virus vaccines (Table 7)(7). Their clinical histories showed that all seven had immediate-type reactions (e.g., systemic urticaria, angioedema, lip swelling, wheezing and cough, airway obstruction, or vomiting), upon ingesting gelatin-containing foods. Five of the seven children showed the immediate-type reactions to food gelatin within 1 month after the vaccination. It therefore appeared that the vaccination triggered the subsequent onset of food-allergic reactions to gelatin.

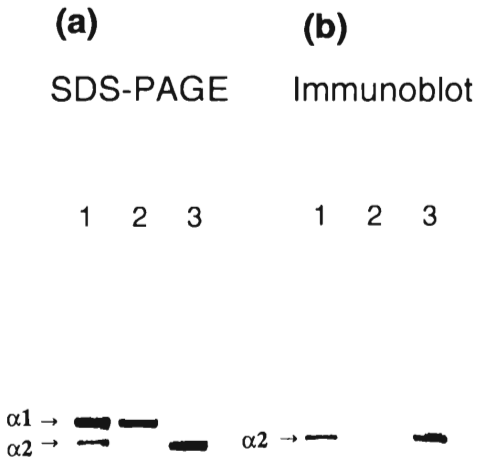

Fig. 2. Analysis of SDS-PAGE (a) and immunoblotting (b). Lane 1, denatured type I collagen; lane 2, α1 chain; lane 3, α2 chain.

5. Analysis of gelatin allergenicity

Gelatin is prepared by the partial hydrolysis of mainly type I collagen from various animal sources (e.g., bovine and porcine hide and bones) (43). We found that 10 children with anti-gelatin IgE had specific IgE to type I collagen (44). Type I collagen consists of α1 and α2 chains (45). IgE antibodies in their sera reacted with the α2 chain, but not the α1 chain (44). Further, type I collagen preparations showed two bands of α1 and α2 chains by SDS-PAGE (Fig. 2a), and IgE from the pooled serum of three patients reacted only with the α2 chain upon immunoblotting (Fig. 2b). Further, we reported the cDNA coding sequence for the α2 chain of bovine type I collagen (46). The encoded amino acid sequence shows 93% identity to the α2 chain of human type I collagen (47). It was reported that the amino acid sequence of the α1 chain of bovine type I collagen shows 98% identity with the α1 chain of human type I collagen (48,49). These homology data suggest that the α2 chain has higher immunogenicity and allergenicity than the α1 chain. In gelatin allergy, denatured bovine type I collagen is a major allergen, and IgE-binding sites exist in the α2 chain of type I collagen.

We reported the reactivity of IgE in bovine gelatin-sensitive children to gelatins from various animals and the antigenic cross-reactivity between the gelatins (Table 8)(50). Serum samples taken from 10 children who showed anaphylaxis in response to vaccines containing bovine gelatin were tested in this study. The IgE in most of the children reacted to kangaroo and mouse gelatins to which the children had little or no exposure as either a food or a vaccine stabilizer. The IgE binding to kangaroo and mouse gelatins was completely inhibited by bovine gelatin, whereas reciprocal inhibition was not complete, indicating that antigenic cross-reactivity is present between the mammalian gelatins. Most of the children who displayed sensitivity to bovine gelatin showed IgE reactivity to other mammalian gelatins. This reactivity may be primarily due to the antigenic cross-reactivity between mammalian gelatins.

Recently, we found that some patients with fish allergies showed IgE reactivity to fish gelatin (51). There is a cross-reactivity among fish gelatins, but there is little cross-reactivity between fish and bovine gelatins. Fist gelatin (type I collagen) might therefore be a significant allergen in fish allergies.

ACKNOWLEDGMENTS

We are grateful to Dr. Michael Kramer, Infectious Disease

Table 8. Reactivity of anti-gelatin IgE to gelatin from various animals

Gelatin	Children no.									
	1	2	3	4	5	6	7	8	9	10
Bovine	++++[a]	++++	++++	++++	++++	++++	++++	++++	++++	++++
Porcine	++++	++++	+++	++	++	++++	+++	++++	±	–
Kangaroo	+++	++++	++++	++	+	+	+	–	±	–
Guinea pig	+++	++	+++	++	++	+	±	–	++	+
Rat	+++	++	+++	++	+	±	±	±	±	–
Mouse	+++	+++	+++	++	+	±	±	±	–	–
Chick	±	+	–	–	–	–	±	–	+	–
Tadpole	+	±	–	–	+	±	±	–	±	±
Codfish	+++	+	–	–	+	±	±	–	±	–
Salmon	+++	±	±	–	–	+	±	–	+	–
Shark	±	±	–	±	–	–	–	–	+	–
Octopus	±	±	–	+	±	–	±	–	–	–
Ascaris	–	–	±	–	–	–	–	–	–	–

[a] The results are expressed as a percentage of the binding of IgE to each animal gelatin compared with that to bovine gelatin: ++++ ≥100%, +++ ≥75%, 75%> ++ ≥50%, 50%> + ≥25%, 25%> ± ≥5%, – >5%

Surveillance Center, National Institute of Infectious Diseases, Tokyo, for critically reading the manuscript.

REFERENCES

1. Zimmerman, B. and Zimmerman, R. S. (1993): Adverse reactions to vaccines. p.1705-1725. *In* Middleton, E. J., Reed, C. E., Ellis, E. F., Adkinson, N. F., Yunginger, J. W. and Busse, W. W. (eds.), Allergy: Principles and Practice. 4th ed, C.V. Mosby, St. Louis.
2. Businco, L. (1994): Measles, mumps, rubella immunization in egg-allergic children: a long-lasting debate. Ann. Allergy, 72, 1-3.
3. Kelso, J. M., Jones, R. T. and Yunginger, J. W. (1993): Anaphylaxis to measles, mumps, and rubella vaccine mediated by IgE to gelatin. J. Allergy Clin. Immunol., 91, 867-872.
4. Tuttle, J., Chen, R. T., Hadler, S. C., Watson, J. C. and Terracciano, G. J. (1996): Update: vaccine side effects, adverse reactions, contraindications, and precautions. Morbid. Mortal. Wkly. Rep., 45(PR-12), 1-35.
5. Ogura, H., Ogura, Y., Tomoda, Y., Kurashige, T., Sakaguchi, M. and Inouye, S. (1995): Anaphylaxis to measles vaccine in a case with gelatin allergy. Infect. Immun. Childhood, 7, 115-118 (in Japanese).
6. Sakaguchi, M., Ogura, H. and Inouye, S. (1995): Measurement of IgE antibody to gelatin in children with immediate-type reactions to measles and mumps vaccines. J. Allergy Clin. Immunol., 96, 563-565.
7. Sakaguchi, M., Nakayama, T. and Inouye, S. (1996): Food allergy to gelatin in children with systemic immediate-type reactions including anaphylaxis to vaccines. J. Allergy Clin. Immunol., 98, 1058-1061.
8. Kumagai, T., Yamanaka, T., Wataya, Y., Umetsu, A., Kawamura, N., Ikeda, K., Furukawa, H., Kimura, K., Chiba, S., Saito, A., Sugawara, N., Kurimoto, F., Sakaguchi, M. and Inouye, S. (1997): Gelatin specific humoral and cellular immune responses in the children with immediate and nonimmediate type reactions to live measles, mumps, rubella and varicella vaccines. J. Allergy Clin. Immunol., 100, 130-134.
9. Nakayama, T., Aizawa, C. and Kuno-Sakai, H. (1999): A clinical analysis of gelatin allergy and determination of its causal relationship to the previous administration of gelatin-containing acellular pertussis vaccine combined with diphtheria and tetanus toxoids. J. Allergy Clin. Immunol., 103, 321-325.
10. Herman, J. J., Radin, R. and Schneiderman, B. S. (1983): Allergic reactions to measles (rubeola) vaccine in patients hypersensitive to egg. J. Pediatr., 102, 196-199.
11. Aukrust, L., Almeland, T. L., Refsum, D. and Aas, K. (1980): Severe hypersensitivity or intolerance reactions to measles vaccine in six children. Allergy, 35, 581-581.
12. Pollok, T. M. and Morris, J. (1983): A 7-year survey of disorders attributed to vaccination in North West Thames region. Lancet, 1, 753-757.
13. Jantunen-Backman, K., Peltola, H., Backman, A. and Salo, O. P. (1987): Safe immunization of allergic children against measles, mumps, and rubella. Am. J. Dis. Child., 141, 1103-1105.
14. Sakaguchi, M., Yamanaka, T., Ikeda, K., Sano, Y., Fujita, H., Miura, T. and Inouye, S. (1997): IgE-mediated systemic reactions to gelatin included in the varicella vaccine. J. Allergy Clin. Immunol., 99, 263-264.
15. Sakaguchi, M., Yoshida, M., Kuroda, W., Harayama, O. and Inouye, S. (1997): Systemic immediate-type reactions to gelatin including in Japanese encephalitis vaccines. Vaccine, 15, 121-122.
16. Sakaguchi, M., Yoshida, T., Asahi, T., Aoki, T., Miyatani, Y. and Inouye, S. (1997): Development of IgE antibody to gelatin in children with systemic immediate-type reactions to vaccines. J. Allergy Clin. Immunol., 99, 720-721.
17. Sakaguchi, M., Nakayama, T., Fujita, H., Toda, M. and Inouye, S. (2000): Minimum estimated incidence in Japan of anaphylaxis to live virus vaccines including gelatin. Vaccine, 19, 431-436.
18. Takahashi, M., Baba, K., Horiuchi, K., Kamiya, H. and Asano, Y. (1990): A live varicella vaccine. p. 49-58. *In* Lopez, C., Mori, R., Roizman, B. and Whitly, R. J. (eds.), Immunobiology and Prophylaxis of Human Herpes Virus Infections. Adv. Exp. Med. Biol., vol. 278, Plenum Press, New York.
19. Sakaguchi, M., Nakayama, T. and Inouye, S. (1998): Cases of systemic immediate-type urticaria associated with diphtheria-tetanus-pertussis vaccines. Vaccine, 16, 1138-1140.
20. Sakaguchi, M. and Inouye, S.(2000): IgE sensitization to gelatin: the probable role by gelatin-containing diphtheria-tetanus-acellular pertussis (DTaP) vaccines. Vaccine, 18, 2055-2058.
21. Sato, H. (1997): Japanese experience with 60 million doses of acellular pertussis vaccines. p327-329. *In* Brown, F., Greco, D., Mastrantonio, P., Salmaso, S. and Wassilak, S. (eds.), Pertussis Vaccine Trials. Karger, Basel.
22. Kimura, M. and Kuno-Sakai, H. (1990): Development in pertussis immunization in Japan. Lancet, 336, 30-32.
23. Hirohata, H. (1997): Outline of the new immunization low. Modern Physician, 17, 273-275 (in Japanese).
24. Yamanaka, T., Wataya, Y., Kumagai, T., Chiba, C., Ohno, N., Saito, S., Sugawara, Y., Kurimoto, F., Sakaguchi, M. and Inouye, S. (1997): Study on adverse reactions on vaccination. Jpn. Med. J., 3795, 37-41 (in Japanese).
25. Taniguchi, K., Fujisawa, T., Ihara, T. and Kamiya, H. (1998): Gelatin-induced T-cell activation in children with nonanaphylactic-type reactions to vaccines containing gelatin. J. Allergy Clin. Immunol., 102, 1028-1032.
26. Ohsaki, M., Tsutsumi, H., Kumagai, T., Yamanaka, T., Wataya, Y., Furukawa, H., Kojima, H., Saito, A., Yano, S. and Chiba, S. (1999): The relevance of TH1 and TH2 cells in immediate and nonimmediate reactions to gelatin-containing vaccine. J. Allergy Clin. Immunol., 103, 276-281.
27. Miyazawa, H., Saito, S., Kumagai, T., Yamanaka, T., Yasuda, S., Yokota-Tsunetsugu, Y., Inouye, S. and Sakaguchi, M. (1999): IgG antibody to gelatin in children with systemic immediate and non-immediate reactions to measles, mumps and rubella vaccines. Vaccine, 17, 2176-2180.
28. Sakaguchi, M., Miyazawa, H. and Inouye, S. (2000): Sensitization to gelatin in children with systemic non-immediate-type reactions to varicella vaccines. Ann. Allergy Asthma Immunol., 84, 341-344.
29. Andersen, M. M. and Rønne, T. (1991): Side-effects with Japanese encephalitis vaccines. Lancet, 337, 1044.
30. Ruff, T. A., Eisen, D., Fuller, A. and Kass, R. (1991): Adverse reactions to Japanese encephalitis vaccine.

Lancet, 338, 881-882.
31. Nazareth, B., Leven, J., Johnson, H. and Begg, N. (1994): Systemic allergic reactions to Japanese encephalitis vaccines. Vaccine, 12, 666.
32. Tsai, T. F. and Yu, Y. X. (1994): Japanese encephalitis vaccines. p. 671-713. *In* Plotkin, S. A. and Mortimer, E. A. (eds.), Vaccine. W. B. Saunders, Philadelphia.
33. Sakaguchi, M. and Inouye, S. (1998): Two patterns of systemic immediate-type reactions to Japanese encephalitis vaccines. Vaccine, 16, 68-69.
34. Okada, K., Miyazaki, T., Kusuhara, K., Hidaka, Y., Inai, I., Kadoya, A. and Ueda, K. (1995): Adverse reactions after inoculation of DPT vaccines. Relationship between skin rash and constitutes of vaccines. Infect. Immun. Child., 7, 99-102 (in Japanese).
35. Nagel, J., Svec, D., Waters, T. and Fireman, P. (1997): IgE-synthesis in man. I. Development of specific IgE antibodies after immunization with tetanus-diphtheria (Td) toxoids. J. Immunol., 118, 334-341.
36. Matuhasi, T. and Ikegami, H. (1982): Elevation of levels of IgE antibody to tetanus toxin in individuals vaccinated with diphtheria-pertussis-tetanus vaccine. J. Infect. Dis., 146, 290.
37. Cogné, M., Ballet, J. J., Schmitt, C. and Bizzini, B. (1985): Total and IgE antibody levels following booster immunization with aluminum-adsorbed and nonadsorbed tetanus toxoid in human. Ann. Allergy, 54, 148-151.
38. Mark, A., Björkstén, B. and Granström, M. (1995): Immunoglobulin E responses to diphtheria and tetanus toxoids after booster with aluminum-adsorbed and fluid DT-vaccines. Vaccine, 13, 669-673.
39. Maibach, H. I., Dannaker, C. J. and Lahti, A. (1993): Contact skin allergy. p.1605-1647. *In* Middleton, E. J., Reed, C. E., Ellis, E. F., Adkinson, N. F., Yunginger, J. W. and Busse, W. W. (eds.), Allergy: Principles and Practice. C. V. Mosby, St. Louis.
40. Garcia, J. E., Sennt, C., Pascual, C., Fernandez, G., Perez-Carral, C., Diaz-Tejeiro, R., Gomez, T. and Sierva, T. (1993): Anaphylactic reaction to recombinant human erythropoietin. Nephron, 65, 636-637.
41. Sakaguchi, M., Kaneda, H. and Inouye, S. (1999): A case of anaphylaxis to gelatin included in erythropoietin products. J. Allergy Clin. Immunol., 103, 349-350.
42. Wahl, R. and Kleinhans, D. (1989): IgE-mediated allergic reactions to fruit gums and investigation of cross-reactivity between gelatine and modified gelatine-containing products. Clin. Exp. Allergy, 19, 77-80.
43. Kelso, J. M. (1999): Gelatin story. J. Allergy Clin. Immunol., 103, 200-203.
44. Sakaguchi, M., Hori, H., Hattori, S., Irie, S., Imai, A., Yanagida, M., Miyazawa, H., Toda, M. and Inouye, S. (1999): IgE reactivity to $\alpha 1$ and $\alpha 2$ chains of bovine type I collagen in children with bovine gelatin allergy. J. Allergy Clin. Immunol., 104, 695-699.
45. Alberrts, B., Bray, D., Lewis, J., Lewis, J., Raff, M., Roberts, K. and Watson, J. D. (1994): Cell junctions, cell adhesion, and the extracellular matrix. p. 978-979. Molecular Biology of the Cell, 3rd ed. Garland Publishing, New York.
46. Shirai, T., Hattori, S., Sakaguchi, M., Inouye, S., Kimura, A., Ebihara, T., Irie, S., Nagai, Y. and Hori, H. (1998): The complete cDNA coding sequence for the bovine proa2(I) chain of type I procollagen. Matrix Biol., 17, 85-88.
47. de Wet, W., Bernard, M., Benson-Chanda, V., Chu, M. L., Dickson, L., Weil, D. and Ramirex, F. (1987): Organization of the human pro-α(I) collagen gene. J. Biol. Chem., 262, 32-36.
48. Bernard, M. P., Chu, M. L., Myers, J. C., Ramirez, F., Eikenberry, E. F. and Prockop, D. J. (1983): Nucleotide sequences of complementary deoxyribonucleic acids for the pro $\alpha 1$ chain of human type I procollagen. Statistical evaluation of structures are conserved during evolution. Biochemistry, 22, 5213-5223.
49. Bornstein, P. and Traub, W. (1979): The chemistry and biology of collagen. p. 411-632. *In* Naurath, H. and Hill, R. L. (eds.), The Protein. Vol. 4. Academic Press, New York.
50. Sakaguchi, M., Hori, H., Ebihara, T., Irie, S., Yanagida, M. and Inouye, S. (1999): Response of IgE in bovine gelatin-sensitive children to gelatins from various animal. Immunology, 96, 286-290.
51. Sakaguchi, M., Toda, M., Ebihara, T., Irie, S., Hori, H., Imai, A., Yanagida, M., Miyazawa, H., Ohsuna, H., Ikezawa, Z. and Inouye, S. (2000): IgE antibody to fish gelatin (type I collagen) in patients with fish allergy. J. Allergy Clin. Immunol., 106, 579-584.