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

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Background: The number of patients with allergic reactions after administration of gelatin-containing live vaccines is increasingly reported in Japan. These allergic reactions appear to be caused by gelatin allergy. It is still unknown how the patients were sensitized to gelatin.
Objective: To determine the incidence of gelatin allergy and to identify contributing factors to gelatin allergy, we investigated the following clinical aspects: the development of IgE antibodies to gelatin and the relationship of the patients' past history of acellular pertussis vaccine combined with diphtheria and tetanus toxoid (DTaP) to the development of gelatin allergy.
Methods: We evaluated 366 patient reports, submitted from 1994 to 1997, of adverse reactions after immunization with monovalent measles, mumps, and rubella vaccines containing 0.2% gelatin as stabilizer. On the basis of physician reports, the patients were categorized as to the nature of the adverse reaction. We determined the presence of IgE antibodies to gelatin and obtained past immunization history.
Results: The 366 reported patients were categorized as follows: 34 with anaphylaxis, 76 with urticaria, 215 with nonurticarial generalized eruption, and 41 with local reactions only. In 206 patients from whom serum was available, IgE antibodies to gelatin were detected in 25 of 27 (93%) with anaphylaxis, 27 of 48 (56%) with urticaria, and 8 of 90 (9%) with a generalized eruption. None of a group of 41 patients with only local reactions at the injected site and none of a control group of 29 subjects with no adverse reaction had such antibodies. Among 202 patients for whom prior vaccine information was available, all had received DTaP vaccines. Among those for whom the prior DTaP vaccine could be determined to contain gelatin or be free of gelatin, 155 of 158 (98%) subjects had received gelatin-containing DTaP vaccines. This rate is higher than would be expected on the basis of the market share of gelatin-containing (vs gelatin-free) DTaP vaccines (75%). Furthermore, before 1993, when a trivalent measles, mumps, and rubella vaccine (with the same 0.2% gelatin content as the monovalent vaccines) was used and administered before DTaP vaccination, no reports of anaphylaxis to the measles, mumps, and rubella vaccine were received.
Conclusion: Most anaphylactic reactions and some urticarial reactions to gelatin-containing measles, mumps, and rubella monovalent vaccines are associated with IgE-mediated gelatin allergy. DTaP immunization histories suggest that the gelatin-containing DTaP vaccine may have a causal relationship to the development of this gelatin allergy. (J Allergy Clin Immunol 1999;103:321-5.)

Key words: *Gelatin, acellular pertussis vaccine combined with diphtheria and tetanus toxoids (DTaP), anaphylaxis, live vaccines*

Anaphylactic reactions after administration of vaccines were believed to be rare. Formerly, reactions observed after live measles or mumps vaccination had been attributed to either egg proteins or antibiotics.[1] However, Kelso et al[2] reported that a patient with an anaphylactic reaction to measles, mumps, and rubella trivalent vaccine (MMR) had IgE antibodies to gelatin. Monovalent measles and mumps vaccines containing 2% modified gelatin (Haemaccel; Behringwerke, Marburg, Germany) were introduced in September 1993 in Japan. Allergic reactions were subsequently reported, and the measles and mumps vaccines were withdrawn from the market in April 1994.[3] During the 8 months that the vaccines were administered, anaphylaxis was reported at an incidence of 1 in 257,000 doses of measles vaccine and 6 in 28,000 doses of mumps vaccine.[3] Sakaguchi et al[4] also reported that 10 of 11 patients, including these patients,[3] who manifested anaphylaxis after immunization with live measles or mumps vaccines had IgE antibodies to gelatin. Thereafter, additional patients were reported with anaphylaxis to gelatin-containing vaccines.[5,6] Sakaguchi et al[7] also reported that anaphylaxis or urticaria appeared after ingestion of gelatin-containing food among some patients who had anaphylaxis to vaccines.

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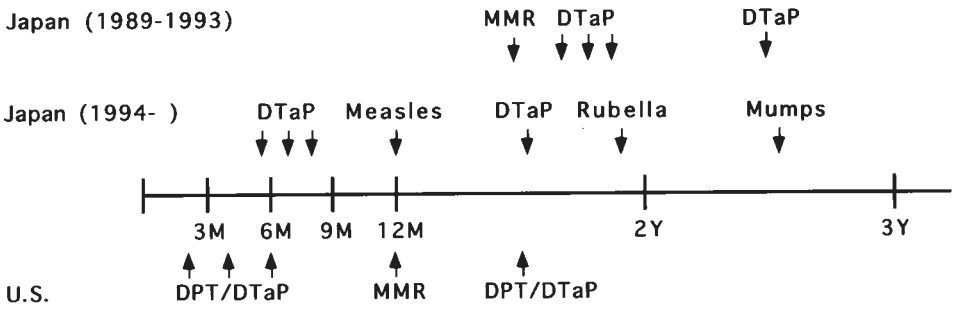

FIG 1. Immunization schedules in Japan and the US.

Abbreviations used
DPT: Diphtheria-pertussis-tetanus
DTaP: Acellular pertussis vaccine combined with diphtheria and tetanus toxoids
MMR: Measles, mumps, and rubella trivalent vaccine

After withdrawal of vaccines containing 2% modified gelatin, the Kitasato Institute started to produce measles and mumps vaccines by using the former stabilizer of 0.2% gelatin, and rubella vaccine also contained the same concentration of gelatin. We investigated the clinical incidence of gelatin allergy to these vaccines on the basis of postmarketing research data.

Alum-precipitated acellular pertussis vaccines combined with diphtheria and tetanus toxoids (DTaP) have been used both for primary and booster immunization since 1981 in Japan. However, no attention has been paid to the immunogenicity of gelatin contained in DTaP vaccine. It should be noted that 4 of 6 manufacturers in Japan added gelatin at a certain stage of the manufacturing process of DTaP vaccine, with final concentrations of gelatin from 48 to 200 μg/mL; 2 manufacturers did not use gelatin in the process. DTaP vaccine contains alum adjuvant to increase the antibody response to toxoids and component proteins. The total market share of the gelatin-containing DTaP vaccine compared with the gelatin-free DTaP vaccine is approximately 75% versus 25% in Japan from the data of official approval doses of vaccine production.

Fig 1 summarizes the difference in immunization schedules between Japan and the US. From 1989 to 1993, most Japanese children had MMR at the age of 18 months and were subsequently immunized with 3 consecutive doses of DTaP. MMR was discontinued in 1993 because of the high incidence of aseptic meningitis related to mumps vaccine strains.[8] The recommended immunization schedule was changed with an amendment to the Preventive Immunization Law of 1994; primary doses of DTaP vaccine were recommended to infants over 3 months of age before live measles vaccination. After 1994, most Japanese children received 3 doses of DTaP from 6 to 10 months of age, measles at 12 months, the fourth DTaP dose at 18 months, rubella around 2 years, and mumps after 2 years. On the basis of the childhood immunization schedule in the US, diphtheria-pertussis-tetanus (DPT) vaccine or DTaP vaccine is recommended at 2, 4, and 6 months of age; MMR at 12 months; and the fourth DPT or DTaP at 18 months.[9]

In this report we present clinical data on allergic reactions to measles, mumps, and rubella monovalent vaccines and the results of the patient's past history of DTaP vaccine immunizations to investigate the possibility that the repeated immunization with gelatin-containing DTaP vaccine induced a sensitization against gelatin before administration of other vaccines.

METHODS

Patients

From May 1994 to December 1997, a total of 366 patients with adverse reactions to live measles, mumps, and rubella monovalent vaccines were reported to the vaccine postmarketing research unit at the Kitasato Institute. A detailed case report was then obtained from the physician, and the case was registered to the Ministry of Health and Welfare. The allergic reactions were classified into 4 categories: anaphylaxis, urticaria, generalized eruption, and local reactions. Anaphylaxis was defined as cases in which the patients demonstrated urticaria or angioedema combined with respiratory symptoms, cardiovascular shock, or both. The second group consisted of those with only urticaria as their reaction. We defined generalized eruption as cases in which the patients had eruptions that were different from urticaria. Local reactions were defined as redness and swelling of greater than 5 cm in diameter at the injected site. Among 366 patients, 41 patients with only local reactions were reported from the same several physicians. We also obtained 29 subjects without adverse reactions after immunization with gelatin-containing live vaccines as a control for the analysis of the presence of IgE antibodies to gelatin by one of the authors. Therefore the subjects of these 2 groups were excluded from the analysis of the background of DTaP immunization history.

IgE antibodies against gelatin

IgE antibodies against gelatin were measured by the method reported by Sakaguchi et al.[4] Briefly, 1 μg of bovine gelatin was coated on the ELISA plate. Anti-human IgE antibody conjugated

with β-galactosidase (Pharmacia AB, Uppsala, Sweden) was added after incubation with serum samples diluted at 1:10 for 3 hours. Then 0.2 mmol/L 4-methylumbelliferyl-β-D-galactoside (Sigma Chemicals, St Louis, Mo) was added as the enzyme substrate, and the fluorescence unit was measured with a fluorometric microplate reader (Fluoroskan II, Labsystems). We considered greater than 0.34 Ua/mL of anti-gelatin IgE antibody as positive.

RESULTS

Nature and timing of adverse reactions

Among 325 patients, 221 demonstrated adverse reactions after live measles vaccine, 45 after live rubella vaccine, and 59 after live mumps vaccine. The details are shown in Table I. Among 325 patients, 34 manifested anaphylaxis (15 after measles vaccine, 9 after rubella vaccine, and 10 after mumps vaccine). The total number of doses shipped of each vaccine was 1.26 million doses of measles vaccine, 1.38 million doses of rubella vaccine, and 0.54 million doses of mumps vaccine during the period of the investigation. The reported incidence of anaphylaxis was 11.9 cases/1 million doses for measles vaccine, 6.52 cases/1 million doses for rubella vaccine, and 18.5 cases/1 million doses for mumps vaccine. An accurate time course for the onset of adverse reactions after vaccination was obtained from 277 patients. The relationship between the symptoms and the onset after vaccination is shown in Fig 2. Most episodes of anaphylaxis (31 of 34) were noted within 30 minutes after vaccination, and urticaria appeared immediately to 24 hours after vaccination. Nonurticarial eruptions and local reactions were observed mostly 3 to 24 hours after vaccination.

TABLE I. Incidence of adverse reaction after live attenuated vaccines

Allergic illness	Measles (n = 221)	Rubella (n = 45)	Mumps (n = 59)	Total (n = 325)
Anaphylaxis	15	9	10	34
Urticaria	52	9	15	76
Eruption	154	27	34	215
Total no of doses shipped	1,260,000	1,380,000	540,000	3,180,000

TABLE II. DTaP vaccine history in patients with adverse reactions

	No of patients			
DTaP history	Measles	Rubella	Mumps	Total
Gelatin+	110 (11)*	23 (7)	22 (6)	155 (24)
Gelatin–	3 (1)	0	0	3 (1)
Unknown	25 (2)	6	13 (3)	44 (5)
DTaP(–)	0	0	0	0
Total	138 (14)	29 (7)	35 (9)	202 (30)

Gelatin+, Gelatin-containing DTaP; *Gelatin–*, gelatin-free DTaP.
*Numbers in parentheses indicate number of patients with anaphylaxis.

IgE antibodies

We examined the presence of IgE antibodies against gelatin in 206 patients, including 41 patients with only local reactions and 29 children without adverse reactions (Fig 3). Among 27 patients with anaphylaxis, 25 (93%) had IgE antibodies against gelatin. IgE antibodies were detected in 27 of 48 (56%) patients with urticaria, and in 25 of 27 patients positive for IgE, urticaria appeared within 3 hours after immunization. In 8 of 90 (9%) patients with generalized nonurticarial eruption, IgE antibodies were detected, and 5 of these patients with positive test results for IgE had symptoms within 3 hours after immunization. In those with local reactions, none had IgE antibodies against gelatin. For a negative control, we examined 29 children without adverse reactions, and all had negative test results for IgE antibodies against gelatin.

DTaP immunization history

Among 325 reported cases of adverse reactions, we obtained information on DTaP vaccination history in 202 cases, and the results are shown in Table II. All patients had a history of 3 or 4 doses of DTaP vaccine. Descriptive information on DTaP manufactures was not obtained for 44 patients; in the remaining 158 patients, information on manufacturers of the DTaP vaccine was available. One hundred fifty-five of these 158 had received gelatin-containing DTaP vaccines. The proportion of patients with adverse reactions to measles, mumps, and rubella monovalent vaccines who had been previously immunized with gelatin-containing DTaP (98%) is significantly higher than the proportion that would be estimated from the data on the market share of gelatin-containing DTaP (75%).

DISCUSSION

Anaphylactic reactions after live measles, mumps, and rubella monovalent vaccines containing gelatin as a stabilizer were associated with IgE-mediated allergy to gelatin in 93% of cases, which is consistent with previous reports.[2,4-6] In this study IgE antibodies to gelatin were also detected in 27 of 48 patients (56%) with urticaria and in 8 of 90 patients (9%) with nonurticarial eruptions. Patients with non-IgE–mediated reactions may have cell-mediated reactions, as reported by Kumagai et al.[5] Another possibility is an arthus-type reaction with anti-gelatin IgG antibodies and immune complexes because some of the patients (previously reported) showed a positive intradermal skin test response against gelatin solution after 4 to 13 hours in accordance with the time course of the onset of generalized eruption, despite showing a negative skin test response after 30 minutes.[10]

In this study the incidence of anaphylaxis was 11.9 cases/1 million doses for measles vaccine, 6.52 cases/1 million doses for rubella vaccine, and 18.5 cases/1 million doses for mumps vaccine from 1994 to 1997. As above, MMR was introduced in 1989 and was discontinued in 1993 in Japan.[8] When we investigated the postmarketing

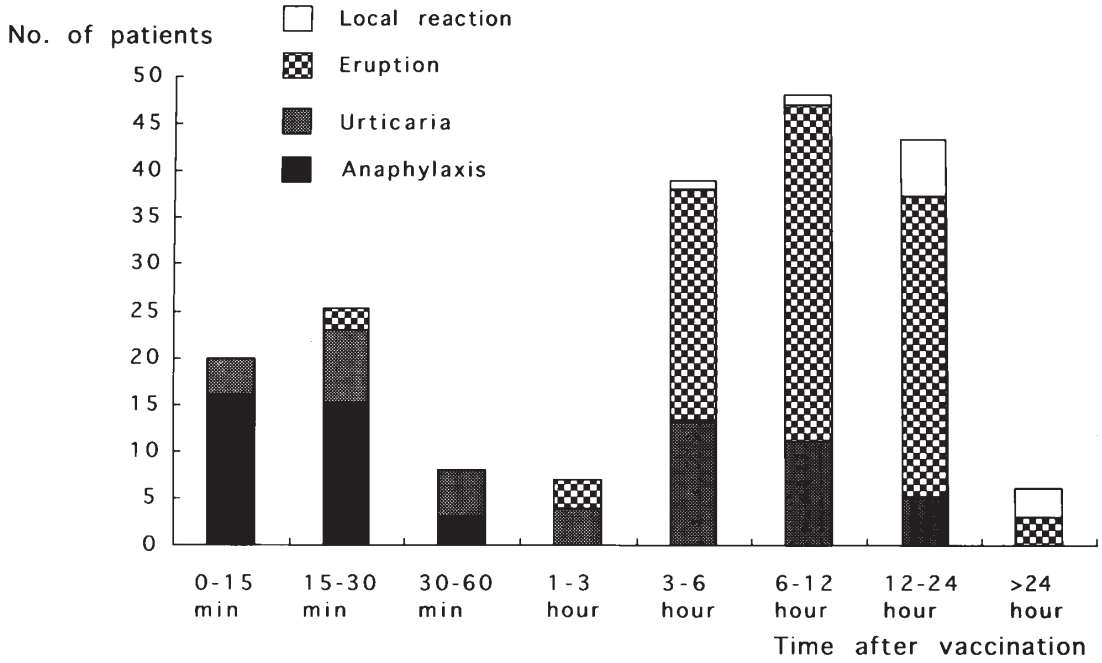

FIG 2. Onset of various allergic symptoms after vaccination.

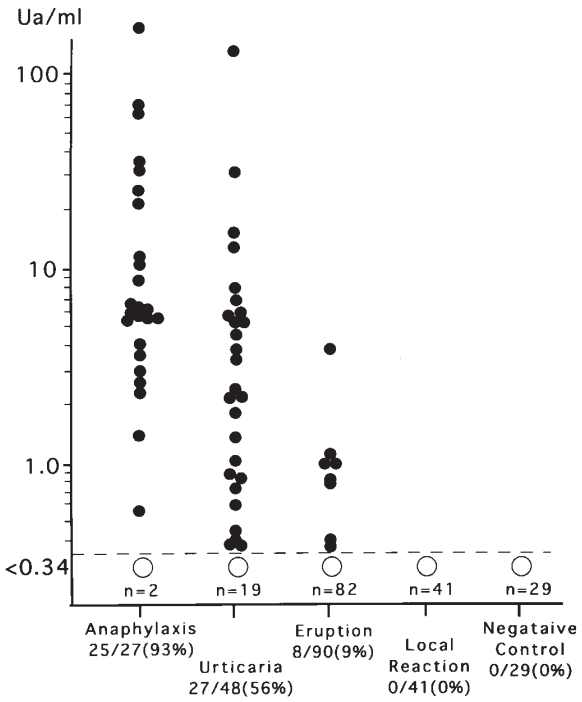

FIG 3. Detection of IgE antibodies to gelatin. Gelatin-specific IgE antibodies less than 0.34 Ua/mL are considered as negative. The numbers of IgE antibodies considered negative are shown under *open circles*. *Filled circles* show individual gelatin-specific IgE antibody levels by the category of adverse reaction or control. The positivity of gelatin-specific IgE antibodies is shown under the categories of adverse reactions.

research during the period from 1989 to 1993, there was no report of anaphylaxis among 974,000 recipients of MMR vaccine produced by the Kitasato Institute despite the fact that the Kitasato Institute used the same lot of gelatin at the same concentration as that used from 1994 on in the monovalent measles, mumps, and rubella vaccines.

It is of great importance to determine why this apparent increase in allergic reactions to vaccines associated with gelatin allergy has occurred. We should consider the possibility of intestinal sensitization by gelatin-containing food materials, but we cannot explain the large difference in the incidence of anaphylaxis only by assuming an increased intake of gelatin-containing food among Japanese infants and children. It was around 1994 that most children started to receive DTaP vaccine before live measles or rubella vaccines, as shown in Fig 1. Reports on anaphylactic reactions after live vaccines began to accumulate at the same time. All of the children who manifested adverse reactions to live vaccines in this study had a history of DTaP vaccination, and among those who had available information on manufacturers of DTaP vaccines, most had received gelatin-containing DTaP vaccines. But our study has a limitation of post-marketing research; only when a recipient had some reactions after vaccination was the case registered. DTaP immunization history was not obtained from an actual control group who received live monovalent vaccines and did not have an adverse reaction at the same study period.

Acellular DTaP vaccine contains alum adjuvant to

enhance the immunogenicity of components of diphtheria toxoid, tetanus toxoid, and elements of pertussis vaccine, such as pertussis toxin and filamentous hemagglutinin, but whole cell DPT vaccine does not. In the preliminary results investigating the immunogenicity of gelatin in DTaP, a trace amount of gelatin in DTaP was immunogenic.[11] We examined 165 paired sera obtained before the first dose of DTaP and 1 month after the third dose of DTaP. Of 165 paired sera, 62 were obtained from the recipients of gelatin-free DTaP, and IgE antibodies to gelatin developed in none. In 103 recipients of gelatin-containing DTaP, IgE antibodies to gelatin developed in 2 recipients. Later, when they received measles vaccine, one developed urticaria 20 minutes after measles vaccination, but the other did not. We should extend the examination to know the rate of sensitization to gelatin through repeated administration of gelatin-containing DTaP.

Although gelatin was thought to be an inert substance, we should consider the possibility of sensitization through repetitive immunization of gelatin-containing DTaP vaccine. DTaP has a low incidence of clinical side effects,[12] and gelatin-free DTaP appears to be desirable for avoiding unnecessary sensitization against gelatin.

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