

A Sero-Epidemiological Survey of Gelatin Sensitization in Young Japanese Children During the 1979–1996 Period

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Abstract

Recently, an increasing number of adverse reactions in children inoculated with live attenuated virus vaccines containing gelatin have been reported. However, the distribution, magnitude and rate of gelatin sensitization in the Japanese population have not been established. Here, the purpose was to investigate the distribution of children with positive gelatin immunoglobulin G (IgG) and/or IgE in Japan and to ascertain whether the incidence of positive antigelatin antibody cases among the general population, as reflected in the sample employed here, has been increasing during the period in question. The presence of IgE and IgG antibodies were measured against gelatin in 1600 panel sera collected from different age groups of Japanese children in Hokkaido/Sapporo from 1979 through 1996. Among the subjects, 39 had gelatin IgG- and/or IgE-positive sera, and these were correlated with the time of obtaining the sera as well as with the age of the subjects. The older the subject and the later the period, the higher the sero-incidence. Japanese children have become increasingly sensitized to gelatin, especially since the mid-1990s.

Introduction

Gelatin was believed to be safe and nonimmunogenic to humans, and therefore has been used as an additive in many vaccine preparations. However, an increasing number of adverse reactions in children inoculated with live attenuated virus vaccines containing gelatin have been reported [1–3]. There are further reports on the pathogenesis and primary sensitization process of this particular allergy [4–8]. Kumagai *et al.* [9] have shown that a series of gelatin-containing diphtheria-tetanus-acellular pertussis (DTaP) vaccinations have caused primary sensitization to gelatin, suggesting that the majority of the Japanese population inoculated with gelatin-containing DTaP vaccine may have been sensitized to gelatin.

Besides the vaccine related issues, there have been concerns about allergic reactions induced by gelatin-containing food [10]. Consumption of gelatin-containing foods in Japan, including jellies, gum and yoghurt as well as ready-made weaning diets has increased dramatically during the last 20 years (Glue & Gelatin Manufacturers Association of Japan and WAKODO, personal communication). This early exposure to gelatin protein and its increased

consumption by infants and young children may have contributed to the increase in gelatin sensitivity. On the basis of the above statement, it may be speculated that dietary gelatin intake has a causal relationship with gelatin sensitization among children.

Irrespective of the primary sensitization process, however, less is known about the incidence of gelatin sensitized subjects among the general population of Japan. The study here was designed to determine the presence of immunoglobulin G (IgG) and/or IgE antibodies to gelatin in panel sera collected from 1979 to 1996 from children to determine whether the incidence of sensitized subjects has increased among infants and young children.

Materials and methods

Sera. Sixteen hundred serum samples collected from 1600 subjects hospitalized at the Hokkaido Children's Hospital and Medical Center or who visited outpatient clinics of private paediatric practitioners in Sapporo from 1979 through 1996 were included in the present study. The study was arbitrarily divided into four periods, 1979–1983 (467 serum samples), 1984–1988 (383 samples), 1989–1992

Table 1 Number of serum samples from each period

Year of sampling	Age (years)						Total
	0	1	2	3	4	5	
1979–1983	129	122	69	55	41	51	467
1984–1988	108	123	50	35	40	27	383
1989–1992	49	53	52	36	27	36	253
1993–1996	74	101	92	81	93	56	497
Total	362	402	263	202	205	166	1600

(253 samples) and 1993–1996 (497 samples). The age distribution of the subjects is summarized in Table 1. The samples had been stored at -20°C until use. Most of the subjects had visited clinics because of respiratory tract infections or diarrheal ailments, were admitted for surgery like inguinal hernia repair, or were being treated for emotional disorders or chronic abdominal pain.

Measurements of gelatin-specific IgE. The fluorometric enzyme-linked immunosorbent assay (ELISA) system was used to determine the IgE antibody to bovine bone gelatin (grade: for biochemistry, Wako Pure Chemical Industries, Osaka, Japan) is similar to the one described elsewhere [11]. Briefly, microplates are coated with 0.1 ml/well of the gelatin solution (10 $\mu\text{g}/\text{ml}$), and after incubation of the serum sample with immobilized antigens, anti-human IgE antibody conjugated with β -D-galactosidase (Pharmacia, Uppsala, Sweden) was added; the enzyme reaction substrate was 0.1 mmol/l of 4-methylumbelliferyl- β -D-galactoside (Sigma Chemical, St. Louis, MO, USA), and the fluorescence (Ex; 355 nm, Em; 460 nm) was measured on a fluorometric microplate reader (f_{max} Fluorescence Microplate Reader; Molecular Devices, Sunnyvale, CA, USA); the antibody value was calculated from a standard curve established by diluting a positive serum that was determined previously; the detection limit of the test was set at 0.05 U/ml for IgE.

Measurement of gelatin-specific IgG. Gelatin-specific IgG antibodies were measured by a photometric ELISA system. Microtitre plates were coated with 0.1 ml/well of the antigen solution (bovine gelatin 10 $\mu\text{g}/\text{ml}$) at 4°C overnight; the plates were washed three times with phosphate-buffered saline with Tween-20 (PBST) and incubated for 2 h at 37°C with 0.3 ml/well of 1% bovine serum albumin in PBST; after washing the plates three times, 0.1 ml/well of serum sample diluted with 1% bovine serum albumin in PBST was added and incubated for 2 h at room temperature; the plates were then washed three times, and 0.1 ml/well of horseradish peroxidase-conjugated goat anti-human IgG (Cappell Product, Durham, NC, USA) was added and incubated for 2 h at room temperature; the enzyme reaction substrate was 1 mg/ml α -phenylenediamine solution (Sigma Chemical) and 0.0012% hydrogen peroxide in 0.05 M citrate buffer (pH 5.0); 0.1 ml/well of substrate solution was added to the plates, which was then incubated at 37°C for 15 min

in the dark. The reaction was stopped by the addition of 0.1 ml/well of 1 M sulfuric acid, and the absorbance (at 492 nm) was measured on a photometric plate reader (Toso, MPRA4I, Japan). The levels of specific antibodies were represented as arbitrary units per millilitre. The arbitrary units per millilitre of each serum was calculated from standard titration curves of a pooled serum known to have highly positive gelatin IgG. The positive/negative cut-off point in the gelatin IgG level was determined by an assay of 35 specimens from subjects who had never received gelatin-containing vaccines. The age distribution of these subjects was from 5 to 24 months. The standard deviation of the data was calculated, and a mean value greater than $+3\text{SD}$ was adopted as the cut-off point. The arbitrary units per millilitre of 0.10 was considered to be positive. The mean arbitrary unit per millilitre and its standard deviation were 0.029 and 0.023, respectively, and thus the arbitrary value of 0.10 was 3.09 SD from the mean value.

Statistical analysis. All statistical analyses were performed using JMP statistical software (SAS Institute, Cary, NC, USA). Logistic regression analysis was used to determine a crude odds ratio to compare the incidence of positive gelatin antibodies. Determination of the age-adjusted odds ratio was performed by multiple logistic regression analysis and the 95% confidence intervals was evaluated.

Results

As shown in Tables 2 and 3, only one of the 467 sera had antigelatin IgG in the 1979–1983 sample, and there was no IgE-positive serum in this period. In the 1984–1988 period, three out of 383 sera had positive IgG, and two of these also had gelatin IgE. In the 1989–1993 period, 10 out of 253 sera had gelatin IgG and five of these also had gelatin IgE, one serum sample had only gelatin IgE and not IgG in this period. In the 1993–1996 period, 23 of 497 sera had gelatin IgG and eight of these also had gelatin IgE, one sample had only gelatin IgE. Thus, a total of 39 samples had gelatin IgG and/or IgE among the subjects investigated in this study. In the following analysis, serum samples with gelatin IgG and/or IgE are regarded to be from subjects with gelatin antibody. The incidence of

Table 2 Odds ratio of positive antigelin immunoglobulin G (IgG) antibody cases divided by sampling year

Year of sampling	Number of tested sera	Number of IgG-positive sera	Crude odds ratio (95% CI)	Age-adjusted odds ratio (95% CI)
1979–1983	467	1	1.00	1.00
1984–1988	383	3	3.68 (0.47–74.5)	4.02 (0.51–81.8)
1989–1992	253	10	19.18 (3.64–352.8)	17.37 (3.28–320.2)
1993–1996	497	23	22.61 (4.74–405.2)	19.77 (4.12–354.8)

positive gelatin IgG and IgE in each period in accordance with the age of the subjects is shown in Fig. 1.

The first period (i.e. 1979–1983) was used as a reference, and a crude odds ratio of the incidence of IgG-positive samples in the following periods was determined with logistic regression analysis (Table 2). This data show that irrespective of age, the incidence of subjects with gelatin antibody increased throughout the period studied here. The age-adjusted odds ratios of 17.37 in the period of 1989–1992 and 19.77 in the 1993–1996 period were statistically significantly different from the first period (1979–1983). Because the number of IgE-positive sera in the whole sample studied here was small, and there were no cases in children younger than 1 year, IgE-positive sera in only sera from children older than 1 year of age were included, and these were divided into two periods (1979–1986 and 1989–1996). As shown in Table 3, the age-adjusted odds ratios of the later to the earlier period was 7.19, and the difference is statistically significant.

Discussion

The present study shows that the incidence of gelatin sensitization among children in Japan comprising the sample here, based on the presence of serum gelatin IgG and/or IgE, has been increasing during the 1979–1996 period. For most of the long history of the Japanese, nutrient protein was predominantly obtained from grain and soybeans, and only in recent decades have the Japanese started eating animal protein such as beef and pork that contain gelatin. This change in dietary habits is dramatic and has occurred in a relatively short period of time. This circumstance may explain why case reports of gelatin hypersensitivity are concentrated in Japan [12], although a number of patients with gelatin hypersensitivity were recently reported from Finland [13]. Further, gelatin-containing vaccines that may also be a factor in the accumulation of gelatin allergy have come into widespread use recently. Today, Japanese vaccine manufacturers have eliminated gelatin from DTaP vaccine products, whereas gelatin

removal from live vaccines has been delayed due to technical difficulties. Thus, there has been an interval between the removal of gelatin from DTaP vaccine and its removal from live viral vaccines, and some children in Japan have received gelatin-free DTaP vaccine, followed by vaccination with gelatin-containing live viral vaccines. This may explain why the incidence of clinical reactions caused by gelatin hypersensitivity has declined during the same period, suggesting that gelatin-containing DTaP vaccine plays a role in the sensitization process. However, it must be noted that acellular DTaP vaccine, 75% of which contain gelatin, was introduced in Japan in 1981, whereas the increase in the number of patients with gelatin hypersensitivity began around the early 1990s, far later than the introduction of acellular DTaP vaccine. Thus, all the known forms of gelatin besides that contained in vaccines should be considered as potential stimulating antigens. Gelatin contained in food has been seen as a prime candidate to sensitize the general population of Japan, as the food gelatin consumption has increased rapidly. Alternatively, the clinical experience that the occurrence of clinical reactions elicited by gelatin has remarkably decreased in the recent few years argues against a significant role for food gelatin as an antigen-eliciting primary sensitization related to gelatin hypersensitivity as the increase in consumption of food gelatin has continued during this period (personal communications). These considerations coincide with the report by Ozaki *et al.* that clinical reactions to varicella vaccine, which could be attributed to gelatin hypersensitivity, have increased during the period 1994–1996. It is unknown why the occurrence of gelatin-related events have been concentrated in the 1994–1996 period in spite of the fact that varicella vaccine has contained gelatin since 1974. A more detailed analysis of specific cases of gelatin hypersensitivity would be necessary to explore whether other factors have been involved in the increase in gelatin hypersensitivity, although the small number of subjects of whom detailed information is available limits epidemiological approaches such as multivariate analysis.

Table 3 Odds ratio of positive antigelin immunoglobulin E (IgE) antibody cases divided by sampling year for subjects more than 1 year old

Year of sampling	Number of tested sera	Number of IgE-positive sera	Crude odds ratio (95% CI)	Age-adjusted odds ratio (95% CI)
1979–1988	613	2	1.00	1.00
1989–1996	627	15	7.49 (2.10–47.6)	7.19 (2.00–45.9)

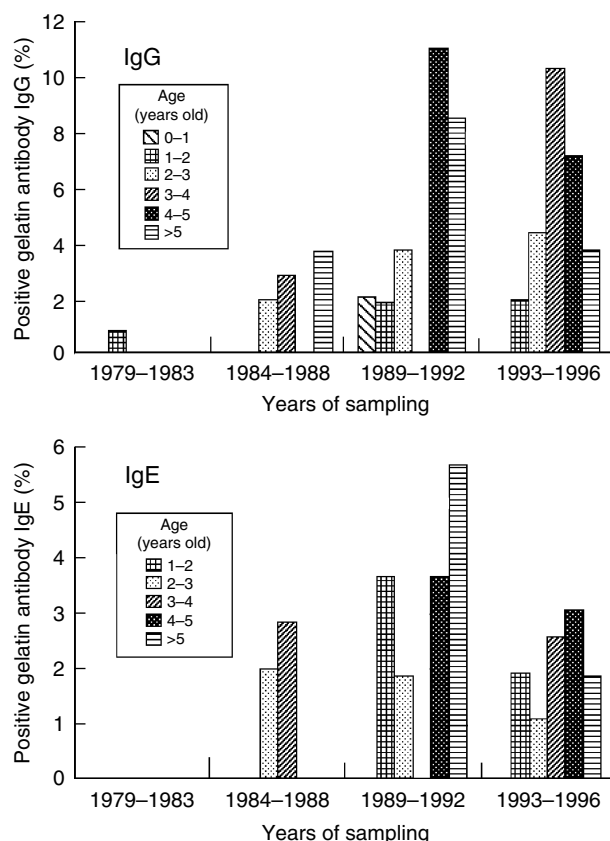


Figure 1 Incidence of positive anti-gelatin immunoglobulin G (IgG) and IgE in each period by the age of subjects. The abscissa shows the sampling periods, and the ordinate is the percentage of subjects who had gelatin IgG and IgE among all the tested subjects.

Caution should be used in interpreting the results here. There is a lack of information of the vaccination and dietary history of the children with antigelatin antibodies as well as some imbalance in the age distribution of the subjects. Also, there were some years when no sera could be obtained. Thus, it may be questioned whether the panel sera used in the present study represent the general population of children in Japan. Because of these limitations, the data here do not offer a conclusive explanation of the mechanisms through which sensitization to gelatin has occurred among the subjects of the present study.

Still, the findings in this report provide information for a better understanding of existing issues related to gelatin hypersensitivity. It is vital for all involved in immunization programs, policy regulations, and vaccine manufacture in North America and Europe, where acellular DTaP vaccine has been introduced recently, to understand this issue. Further study is necessary to monitor the status of sensitization to gelatin in countries other than Japan and to explore whether similar problems may have occurred elsewhere such as reported from Finland. In Japan, low allergenic gelatin has been developed for medical preparations including vaccines and cytokines in an effort to develop safe colloidal

substances as a replacement for conventional gelatin [11, 14, 15]. There have also been efforts to improve dietary gelatin in terms of its allergenicity, utilizing starting materials other than cow bone or porcine skin (personal communication).

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