

Gelatin-containing diphtheria–tetanus–pertussis (DTP) vaccine causes sensitization to gelatin in the recipients

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Abstract

Gelatin-specific T cell response was performed to determine whether a series of vaccinations with gelatin-containing DTP is a primary sensitization process in gelatin allergy. Thirty-seven recipients with gelatin-containing DTP who developed adverse reactions after vaccination and eight recipients of DTP without gelatin who also developed adverse reactions were studied. In addition, 10 subjects receiving gelatin-containing vaccine and 10 subjects inoculated with non-gelatin vaccine who did not show any adverse reactions were also investigated. All subjects inoculated with gelatin-containing DTP vaccine showed positive T cell responses against gelatin, however, occurrence of adverse reactions did not correlate with T cell responses. We conclude that DTP vaccine containing gelatin induces sensitization to gelatin in the recipients, but the mechanism of local reactions remains unknown. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Recently, an increasing number of adverse reactions, including anaphylaxis, have been reported in infants and children after inoculation with gelatin-containing vaccine [1–3]. Gelatin-specific IgE was detected in the serum of patients experiencing immediate reactions [4–6]. In addition, more than 80% of patients with immediate reactions, as well as those with nonimmediate reactions, responded positively to a gelatin-specific lymphoproliferation test [7], suggesting that cell-mediated immunity is important in this newly

recognized allergic reaction [8]. However, how and when the subjects were sensitized to gelatin is unknown.

In Japan, six companies manufacture diphtheria–tetanus–pertussis (DTP) vaccine and only one company deliberately adds gelatin to the product. Although it was believed that gelatin was not contained in the DTP vaccine produced by the other five companies, recently, gelatin was identified in the DTP vaccine made by three of these five. This gelatin was a contaminant carried over from the early stages of manufacture. Moreover, DTP vaccine also contains aluminum adjuvant that may increase immunological responses against gelatin. Thus, there is a valid concern that gelatin in DTP vaccines may sensitize the recipients and cause future clinical symptoms due to gelatin allergy.

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Table 1
Characteristics of the subject

Subjects No.	Gelatin in DTP	Adverse reaction	Gender		Age (m(±S.D.))
			male	female	
37	(+)	(+)	24	13	11.2 (±7.2)
10	(+)	(–)	5	5	15.0 (±4.1)
8	(–)	(+)	3	5	13.5 (±6.3)
10	(–)	(–)	4	6	10.4 (±3.0)

This study was designed to examine the possibility that a series of DTP vaccinations caused primary sensitization, and in addition, whether gelatin-containing DTP vaccine inoculated into recipients who did not show any adverse reactions might also result in gelatin sensitization.

2. Materials and methods

2.1. Subjects

The study was conducted from April 1997 to May 1998 at four pediatric outpatient clinics. One hundred and fifty-three and 38 infants and children were vaccinated with a series of three doses of DTP vaccine containing or not containing gelatin, respectively. Thirty-seven of the 153 recipients (24.2%) given gelatin-containing DTP and eight of the 38 recipients (21.1%) of DTP without gelatin who developed adverse reactions to vaccination were enrolled. In addition, 10 subjects inoculated with gelatin-containing vaccine and 10 subjects given non-gelatin vaccine who did not show any adverse reactions were also included. Adverse reactions consisted of redness, swelling and induration at the injection site which were sharply demarcated, exceeded 1 cm in diameter, appeared more than 12 h after administration of vaccine and persisted for a few days. Transient flushing and redness appearing just after administration of vaccine was excluded from this category. Only one patient developed a mild systemic cutaneous reaction which was composed of a measles-like military rash without itching and disappeared without any pigmentation within a couple of days. Nobody developed an immediate-type reaction such as urticaria or respiratory symptoms. Informed consent was obtained from the parents of the patients after the project was thoroughly explained. The subjects were carefully examined every day by the parents and physicians for 7 days after vaccination. In order to clarify the time-course of the lymphocyte activity, we collected blood samples at least twice in six subjects at varied time intervals, (4–30 days), after the onset of the reaction.

2.2. Determination of gelatin-specific IgE antibody

IgE antibody to bovine gelatin (Wako Junyaku, Tokyo, Japan) was measured by a fluorometric ELISA system. Microplates were coated with bovine gelatin (10 µg/ml). After incubation of the serum sample with immobilized antigens, anti-human IgE antibody conjugated with β-D-galactosidase (Pharmacia, Upsala, Sweden) was added. The enzyme reaction substrate was 0.1 mmol/L 4-methylumbelliferyl-β-D-galactoside (Sigma Chemical Co., St. Louis, MO). The fluorescence unit was measured on a fluorometric microplate reader (f max fluorescence microplate reader; Molecular Devices, Sunnyvale, CA).

2.3. In vitro lymphocyte proliferation test (LPT)

The in vitro lymphocyte proliferation test used resembles one described previously [7], except that autologous serum instead of pooled AB+ serum was used to prepare the culture medium. The positive/negative cut-off level value was determined on the basis of comparison with the distribution of the SI values obtained with more than 30 subjects who never had received gelatin-containing vaccines. Standard distribution of obtained data was calculated and mean value + 3S.D. was considered to be the cut off point. A stimulation index ≥1.5 was considered to be a positive response.

2.4. Antigen-specific interleukin 2 responsiveness (AIR)

The technique has been described previously [9]. An SI increment of 0.08 or more, which is equivalent to 2.45S.D. above the mean value obtained in 14 subjects who never had received gelatin-containing vaccines, was regarded as a positive response.

2.5. Statistical analysis

Characteristic values in the groups of subjects were compared by one way analysis of variance. The Mann–Whitney *U* test and Kruskal–Wallis test were utilized for quantitative variables expressed as the stimulation index in the lymphoproliferation assay and

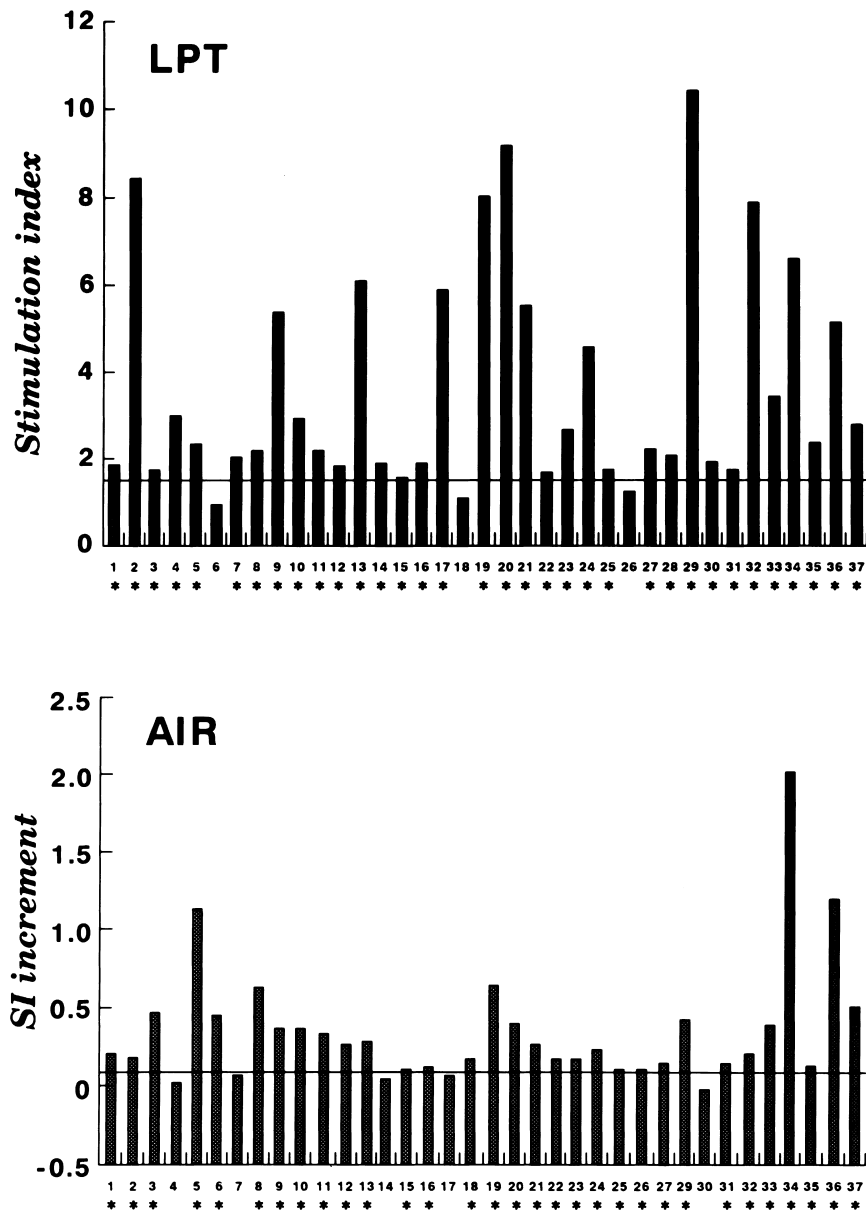


Fig. 1. Gelatin-specific lymphoproliferation response (top) and IL-2 responsiveness (bottom) among recipients of gelatin-containing vaccine with adverse reactions. The horizontal line defines the positive negative cut off point while asterisks identify subjects with positive responses.

as stimulation index increment in the assay for IL-2 responsiveness. Fisher's exact test was used to examine the association between categories of positiveness in gelatin-specific cell-mediated immunity and those of the presence of gelatin in DTP vaccine inoculates. All *P*-values are two-sided. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of subjects

The demographic data of subjects are shown in

Table 1. We did not mandate the physician's choice of DTP vaccine manufacturer in each outpatient clinic. Therefore, the proportions of DTP vaccine with or without gelatin roughly reflect the market share in the recipient population in Japan and were not equal among the study groups. In addition, all of the subjects with adverse reactions agreed to blood sampling, whereas only a few subjects without adverse reactions were recruited to the study. These factors resulted in an imbalance in the number of subjects in each study group. There was no difference in age distribution in each group of recipients. Among 37 recipients of gelatin-containing vaccine, adverse reactions appeared after the 1st vaccination in three (8%) cases, and after

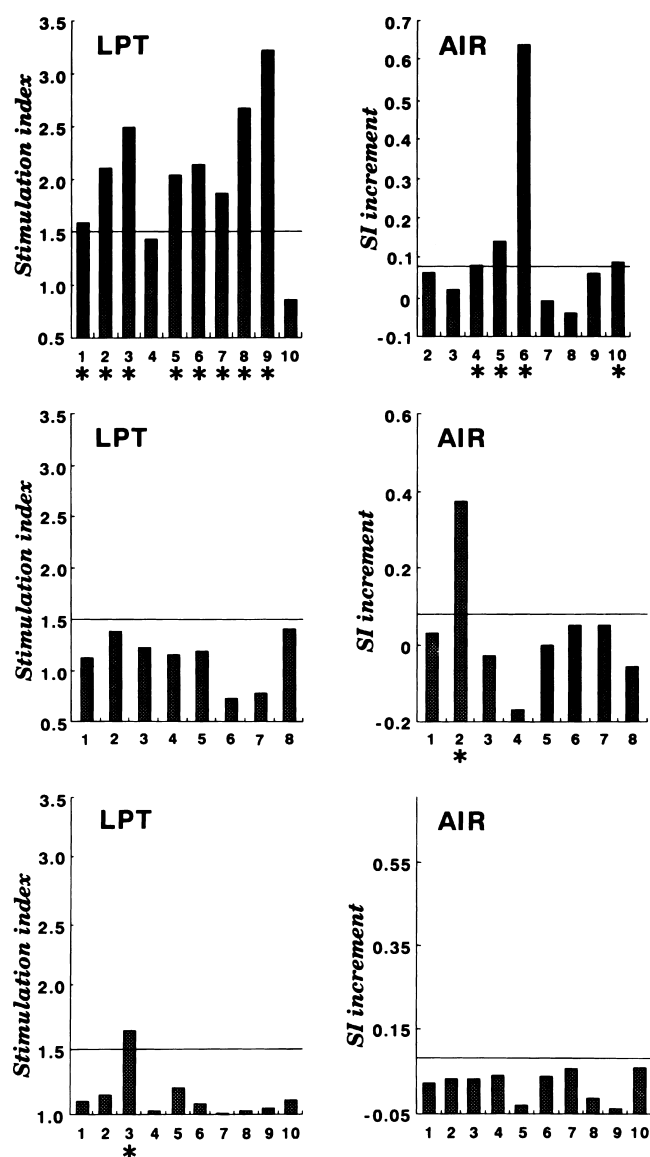


Fig. 2. Gelatin-specific lymphoproliferation response and IL-2 responsiveness in recipients of gelatin-containing vaccine without adverse reactions (top), in recipients inoculated with non-gelatin DTP who reacted adversely (middle) and those who did not react adversely (bottom). The horizontal line shows the positive negative cut off point and asterisks identify subjects with positive responses.

the 2nd and 3rd vaccinations in 16 (43%) and 18 (49%) cases, respectively. Among eight recipients of DTP without gelatin, adverse reactions appeared after the 1st vaccination in two (25%) cases, and after the 2nd and 3rd vaccinations in three (38%) cases each. In both of the above groups, the adverse reactions after the 1st vaccination occurred several days after inoculation, whereas those after the 2nd and 3rd vaccinations appeared by the next day or the subsequent day after inoculation.

Serial measurements in six recipients showed that the maximal response in the gelatin-specific cell mediated immune reaction in each subject generally appeared in the 2nd week after the adverse reactions developed (data not shown), therefore we collected blood samples from each subject 2 weeks after the adverse reaction onset. Samples were not necessarily collected after completion of a full series of three doses of DTP in all subjects. Almost half of the specimens from subjects with adverse reactions were collected following the 1st or 2nd vaccination. Specimens from subjects inoculated with DTP with or without gelatin who never developed any adverse reactions were collected 2 weeks after vaccination.

3.2. IgE antibody specific to gelatin

None of the subjects, inoculated with DTP containing or not containing gelatin, had positive IgE responses.

3.3. Gelatin-specific cell-mediated immune responses

Among 37 recipients of gelatin-containing vaccines with adverse reactions (Fig. 1), 34 (92%) had a positive LPT. Of 36 subjects investigated with AIR, 31 showed a positive response (86%). Twenty-eight cases were positive in both LPT and AIR, five and three patients were positive only in LPT and AIR, respectively. All subjects in this group reacted positively to either both tests or one of LPT and AIR.

Of 10 recipients of gelatin-containing vaccine without adverse reactions (Fig. 2 top), 8 (80%) showed a

Table 2
Gelatin-specific cell-mediated immune responses in subject groups

Subjects No.	Gelatin in DTP	Adverse reaction	No. positive (%) in		Test results					No. positive (%) in CMI
			LPT	AIR	LPT	(+)	(+)	(-)	(-)	
					AIR	(+)	(-)	(+)	(-)	
37	(+)	(+)	34 (92%)	31/36 (86%)	28	5	3	0		37 (100%)
10	(+)	(-)	8 (80%)	4/9 (44%)	2	5	2	0		10 (100%)
8	(-)	(+)	0 (0%)	1 (13%)	0	0	1	7		1 (13%)
10	(-)	(-)	1 (10%)	0 (0%)	0	1	0	9		1 (10%)

positive response in LPT. Only nine subjects were investigated with AIR of whom four were positive (44%). Two patients were positive to both LPT and AIR, while five and two cases responded positively only in LPT and AIR, respectively. All subjects in this group were either positive to both or one of LPT and AIR.

Among recipients inoculated with DTP without gelatin, 1 (13%) of eight recipients who developed an adverse reaction (Fig. 2 middle) and 1 (10%) of 10 recipients without an adverse reaction were positive in AIR and LPT (Fig. 2 bottom), respectively.

Subjects inoculated with gelatin-containing vaccine who developed adverse reactions when compared with those who did not, showed no significant difference in the stimulation index in the lymphoproliferation assay (3.653 ± 2.571 vs. 2.181 ± 0.557 , $P = 0.23$), although a significant difference in stimulation index increment was observed (0.342 ± 0.394 vs. 0.119 ± 0.218 , $P = 0.0074$). Therefore, we divided the subjects into two groups, one being patients with adverse reactions and the other those without adverse reactions. Table 2 summarizes the gelatin-specific cell-mediated immune responses in the various subjects as detected by the lymphoproliferation test and IL-2 responsiveness assay. Of the adverse reaction patients, the prevalence of positive gelatin-specific cell-mediated immune responses was significantly greater among those receiving gelatin-containing DTP vaccine than among those inoculated with gelatin free DTP vaccine (100 vs. 13%, $P < 0.0001$). Among the subjects without adverse reactions, the prevalence of a positive gelatin-specific cell-mediated immune response was significantly greater among the subjects given gelatin-containing DTP vaccine than among those receiving gelatin free DTP vaccine (100 vs. 10%, $P = 0.0001$).

In order to investigate whether higher activities in LPT and AIR were observed after the second or third DTP vaccination than after the first DTP vaccination, we compared the data in LPT and AIR in accordance with the frequency of vaccination. There was no statistically significant relationship between frequency of vaccination and LPT and AIR activities ($P = 0.35$ and 0.19 , respectively).

4. Discussion

Adverse reactions in children inoculated with gelatin-containing vaccines are increasing [3], and gelatin is a recognized cause of immediate and non-immediate reactions with clinical symptoms ranging from anaphylaxis to mild skin eruptions and swelling at the local injection site [4,10–13]. Cell-mediated immunity is crucial in the pathogenesis of these reactions [7]. However, the process of primary sensitization remains

unknown. For years a significant number of DTP vaccine recipients have developed local swelling and redness, sometimes with induration, which physicians have usually attributed to DT toxoid. We suspected that the gelatin in DTP vaccines might be the cause of primary sensitization.

The exact concentration of gelatin in each DTP vaccine is unknown. Some information regarding the amount of gelatin contained in each DTP vaccine product is provided in the manufacturer's literature which gives the contents of the vaccine and the volume of vaccine administered. It is stated that each DTP vaccine dose contains several to several tens micrograms of gelatin. In addition to this information provided by the vaccine manufacturers, an assay study suggests that the amount of gelatin present is around 5–15 µg/dose and varies between vaccine brands. These data were obtained by an inhibition assay system utilizing gelatin-specific IgE, however, the gelatin detected by this assay is only that in water soluble form. Most gelatin contained in DTP vaccine is trapped and surrounded by alum adjuvant and is not detected by an inhibition assay (Nakayama T: personal communication). Thus, the total amount of gelatin contained in DTP vaccines was estimated by calculating from the basic materials used and the dilution factor of the manufacturing process. Based on this it is around 20 to 100 µg/dose [14].

We established an assay system to investigate gelatin allergy in vaccine recipients which detects sensitization to gelatin at the cellular immune level. The lymphocyte proliferation test is sufficiently sensitive to detect the activation of lymphocytes in patients not yet producing gelatin-specific IgE. Kuno-Sakai et al. [15] reported that gelatin-specific IgE was generated in only one of 24 subjects inoculated with a series of gelatin-containing DTP vaccine. Thus, it is inappropriate to base the diagnosis of early sensitization to gelatin on a positive gelatin-specific IgE [7]. Recently, Ohsaki et al. [16] studied the basic mechanism of lymphocyte responses to gelatin by analysis of cytokine messenger RNA. Their findings provide additional support to the use of the lymphocyte proliferation assay for the investigation of gelatin allergy.

Our data clearly show that, regardless of the occurrence of adverse reactions, all subjects inoculated with gelatin-containing DTP vaccine developed positive T cell responses against gelatin, compared to less than 13% of all recipients of DTP vaccine without gelatin. It is surprising that a minute quantity of gelatin should prime and establish a gelatin allergy in 100% of recipients, even though adjuvant was added as an ingredient. Notably some recipients showed a positive response even after a single inoculation with gelatin-containing vaccine. This suggests that the vast majority of the Japanese population inoculated with gelatin-

containing DTP vaccine have been sensitized to gelatin. The primary cause of sensitization directed against gelatin among subjects inoculated with gelatin free DTP and who showed positive responses in the lymphoproliferation assay remains unknown. Dietary gelatin intake may be a factor. This positive rate of 10–13% in the general population, even in sensitization at the lymphocyte level, will pose another big issue to allergologists beside vaccine-induced allergy.

Recently, Nakayama et al. [14] reported their clinical observation which suggests a causal relationship between DTP immunization and the development of gelatin allergy. The results presented here provide direct laboratory-based evidence that vaccination with DTP-containing gelatin causes universal sensitization in the general population and raises the possibility that some recipients will generate gelatin-specific IgE and be at future risk for a wide range of clinical reactions including anaphylaxis. In terms of absence of specific IgE reactions in the present study population, we believe it is because the subjects were still in an early phase of sensitization in which only T cells were sensitized to gelatin and recognized it *in vitro* while production of gelatin-specific IgE occurs later. In this early phase of sensitization the risk of systemic reactions may be low in the event of the intake of gelatin parenterally or orally, however, repeated intake of gelatin in such subjects may elicit progressively greater boosts to the immune responses predisposing to future severe reactions. Primary care physicians should be aware of this risk and be ready to deal with adverse reactions, including anaphylaxis, caused by another vaccination or by the intake of food-containing gelatin. In addition, our findings have special importance for officials who have a primary responsibility to control biological products in many nations and for vaccine manufacturers who develop and market vaccines. Our results also suggest that the unnecessary addition of gelatin to biological products such as acellular pertussis vaccine should be avoided, and if gelatin must be used, such as in the preparation of cytokine products or other medications [17], the improved gelatin called “FreAlagin” recently developed in Japan might be considered [18]. FreAlagin is low molecular weight fraction of porcine gelatin produced by a process of enzymatic digestion and shown to be non-antigenic and of low-allergenic potential. We have also tested the stimulation activity of FreAlagin to sensitized lymphocytes employing the lymphocyte proliferation assay (manuscript submitted).

Our observation in this study that the frequency of adverse reactions appeared to be no greater in those who received gelatin-containing vaccines (24.2%) compared with those who received gelatin-free vaccines (21.1%) suggested symptoms appearing after DTP vaccination are not necessarily related to gelatin allergy.

Local redness and swelling at least caused by DTP vaccinations may not be immune mediated as suggested by Kelso [19].

As to why almost all reports related to gelatin allergy are from Japan, a role for ethnic background, possibly relating to HLA types has been proposed by Kelso [19]. Recently we undertook HLA typing experiments utilizing specimens of subjects with gelatin allergy and have obtained interesting results (manuscript in preparation).

Some questions still remain unanswered. Which population of recipients, with established sensitization caused by DTP with gelatin, will generate IgE in the future? How long does sensitization caused by gelatin-containing DTP vaccination persist? Studies related to these issues are in progress.

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