

Vaccine adverse events reported in post-marketing study of the Kitasato Institute from 1994 to 2004

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Abstract

General physicians, pediatricians and parents realize that serious adverse events occur with an extremely rare incidence, but have no information on the incidences of vaccine-associated adverse events. A proper understanding of vaccine adverse events would be helpful in promoting an immunization strategy. Causal association can rarely be determined in adverse events through laboratory examinations. We examined the cases reported in the post-marketing surveillance of the Kitasato Institute, categorizing them into two groups: allergic reactions and severe systemic illnesses. Anaphylactic patients with gelatin allergy after immunization with live measles, rubella and mumps monovalent vaccines have been reported since 1993, but the number of reported cases with anaphylaxis dramatically decreased after 1999 when gelatin was removed from all brands of DPT. The incidence of anaphylactic reaction was estimated to be 0.63 per million for Japanese encephalitis virus (JEV) vaccine, 0.95 for DPT and 0.68 for Influenza vaccine, but the causative component has not yet been specified. Among 67.2 million immunization practices, 6 cases with encephalitis or encephalopathy, 7 with acute disseminated encephalomyelitis (ADEM), 10 with Guillain–Barré syndrome and 12 with idiopathic thrombocytopenic purpura (ITP) were reported. The wild-type measles virus genome was detected in a patient with encephalitis and in two of four bone marrow aspirates obtained from ITP after measles vaccination. Enterovirus infection was identified in two patients after mumps vaccination (one each with encephalitis and ADEM), one patient with encephalitis after immunization with JEV vaccine, and one with aseptic meningitis after immunization with influenza vaccine. The total estimated incidence of serious neurological illness after vaccination was 0.1–0.2 per million immunization practices. We found that enterovirus or wild-type measles virus infection was coincidentally associated with vaccination in several cases suspected of being vaccine adverse events.

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1. Introduction

The number of patients with vaccine-preventable diseases has decreased since the introduction of effective vaccines, and many kinds of vaccines are used in developed countries [1]. But, in Japan, acellular pertussis vaccine combined with tetanus and diphtheria toxoids (DPT), inactivated Japanese encephalitis vaccine, live polio vaccine, further attenuated

measles vaccine and rubella vaccines are recommended for routine immunization. Mumps and varicella vaccines are voluntary and the immunization strategy is therefore stagnant. One of the reasons for this is the issue of vaccine safety, and some parents and physicians worry unnecessarily about vaccine adverse events. This has been an obstacle to increasing vaccine coverage and we should therefore clarify this matter to catch up with the worldwide standards [1].

There is no vaccine that is absolutely safe, and subsequently, increased vaccine usage inevitably results in an increased number of adverse medical events associated with

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vaccination. A key issue is to achieve a better understanding of the balanced concept of potential risk, vaccine efficacy, and the incidence of vaccine adverse events. The incidence of adverse events was evaluated at the time of field trials before the licensure of vaccine. It covered only mild well-known reactions, including systemic reactions, such as febrile illness, eruption and local reactions. General physicians, pediatricians, and parents realize that serious adverse events occur but have no information on the incidence of vaccine-associated serious adverse events. A proper understanding will depend upon the post-marketing surveillance (PMS) research. Indistinct information and rumors about serious adverse events influence the popularity of vaccines.

The vaccine adverse event reporting system (VAERS) was developed in the US to increase the capacity to evaluate vaccine safety [2–4]. Approximately 6 million members (2% of the US population) participated and became a valuable resource for information on vaccine safety. Causal association between the vaccine and adverse events can rarely be determined through specific examinations, and false assumptions regarding causality are likely to occur without careful investigation of etiology and pathogenesis [5].

In this study, we analyzed the cases reported in the PMS of the Kitasato Institute and they were classified into two categories: allergic reactions and systemic serious illnesses. We performed virological examinations in the patients who had experienced serious adverse events after vaccination. Virus isolation and genomic diagnosis were performed to detect the corresponding virus species. We also performed the examination to detect the enterovirus genome in samples obtained from sterile sites, such as cerebrospinal fluids, and sera in addition to the virological diagnostic procedures related to the vaccine virus species.

2. Patients and methods

2.1. Data collection and definition

In Japan, physicians who experience cases with serious vaccine-associated events after vaccination should report these to the regional government through the regional Public Health Center. The case is then registered by the Ministry of Health, Welfare and Labor, through legitimate channel, as shown in Fig. 1. Clinical samples are obtained and specific diagnostic examinations are principally carried out at the Regional Institutes for Public Health. Also, the case is reported to the respective vaccine manufacture and should be registered through the private manufacturer's channel. The accuracy of the case identification is confirmed through both channels. In this study, clinical samples obtained by physicians were transferred to our laboratory and clinical data was also obtained from the physicians (Fig. 1).

We summarized the data reported in the PMS Research Unit of the Kitasato Institutes for Biologicals from April 1994

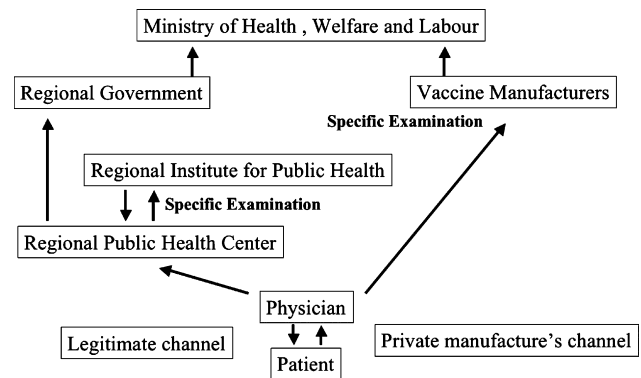


Fig. 1. Reporting system for serious vaccine-associated adverse events after vaccination in Japan.

to December 2004. Reported cases were classified into two groups: allergic reactions and severe systemic illnesses, as shown in Table 1. In the allergic reactions observed within 48 h after vaccination, we classified them into four categories: anaphylaxis, urticaria, eruption, and local reaction. Details of the clinical case definition and co-relation with the presence of IgE antibodies against gelatin were reported previously [6,7]. Briefly, anaphylaxis is defined as the case where the patient demonstrated urticaria or angioedema with respiratory symptoms, cardiovascular shock, or both. The second group consists of the case with only urticaria as their reactions. The third group consists of those with generalized eruption different from nature of urticaria. The fourth, local reactions are defined as redness and swelling greater than 5 cm in diameter. The systemic serious illnesses reported are also shown in Table 1. Criterion of encephalitis and/or encephalopathy is acute onset of fever with neurological symptoms such as seizure, decreased level of consciousness, and positive findings with electroencephalography (EEG) or computerized tomography (CT). Aseptic meningitis is defined the occurrence of a febrile illness with meningeal irritation (headache, vomiting, or stiff neck) with pleocytosis in the CSF ($>15/\text{mm}^3$). Acute disseminated encephalomyelitis (ADEM) is diagnosed by several neurological signs (decreased level of consciousness, visual disturbance, motor

Table 1
Vaccine adverse events

Allergic reactions
Anaphylaxis
Urticarial eruption
Generalized eruption
Severe local reaction
Severe systemic illness
Encephalitis/encephalopathy
Aseptic meningitis
Acute disseminated encephalomyelitis (ADEM)
Guillain-Barré syndrome
Acute cerebellar ataxia
Acute hemiplegia
Idiopathic thrombocytopenic purpura (ITP)
Allergic purpura

neuron disorders, etc.), and by the presence of demyelinating changes in magnetic resonance imaging (MRI) [8]. Guillain–Barré syndrome is diagnosed by clinical symptoms, such as progressive muscle weakness and loss or decreased level of deep tendon reflex [9]. Idiopathic thrombocytopenic purpura (ITP) is diagnosed by a tendency to bleed along with a decreased number of thrombocyte count ($<100,000/\text{mm}^3$).

We produce three kinds of live attenuated measles, rubella and mumps vaccines, and three kinds of inactivated JEV, DPT and Influenza virus vaccines. Live vaccines are packaged in a single dose but inactivated vaccines before 2003 contain two doses per package. We calculated the shipping doses of vaccine as the estimated number of immunization practices.

2.2. Virus isolation and detection of the viral genome

We obtained clinical samples of nasopharyngeal secretion (NPS), cerebrospinal fluid (CSF), peripheral venous blood mononuclear cells (PBMC) or bone marrow aspirates from the patients suspected of having vaccine adverse events. The samples were transferred within 48 h from individual hospitals. B95a cells for measles virus, Vero cells for mumps virus and RK13 cells for rubella virus were used for virus isolation [10–12].

Total RNA was extracted from 200 μl of clinical samples and genomic RNA was transcribed by AMV reverse transcriptase at 50 C for 1 h using virus-specific primers for measles virus, rubella virus, mumps virus, and enterovirus. The measles virus genome was amplified in the nucleocapsid (N) and hemagglutinin (H) regions by RT-PCR, as previously reported [13]. The phospho (P) protein and hemagglutinin-neuraminidase (HN) protein regions of the mumps virus were amplified [14,15], and NS4 and E1 regions of rubella

virus were used for RT-PCR amplification [16]. We designed a nested RT-PCR to detect the 5' non-coding region of enterovirus genome, which is considered to be a relatively conserved region among different serotypes. We reported the detection of enterovirus genome from sera, CSF and NPS, despite being negative for virus isolation [17,18].

2.3. Differentiation between vaccine and wild strains

The measles AIK-C vaccine strain was differentiated from the wild-type strains using restriction fragment length polymorphism (RFLP) of the PCR products of the H gene, which was digested by *Ava* II, *Bst* I, and *Hpa* II [19]. The differentiation of mumps Hoshino vaccine strain was performed using RFLP of the HN gene digested by *Alw* 44 I and *Sca* I, as previously reported [15]. As for the rubella Takahashi strain, PCR products were excised from the gel and the nucleotide sequence was determined with the dye terminator method, using ABI 377 A.

3. Results

3.1. Allergic reactions

Among vaccine adverse events, anaphylactic reaction is rare event, but is sometime serious. From 1993, allergic reactions were increasingly reported after vaccination with live measles, mumps and rubella monovalent vaccines, and are summarized in Fig. 2. After 1999, when the trace amount of gelatin was removed from all brands of DPT, the incidence of gelatin allergy decreased, along with the modification of the gelatin material as a stabilizer [20]. No anaphylactic reaction

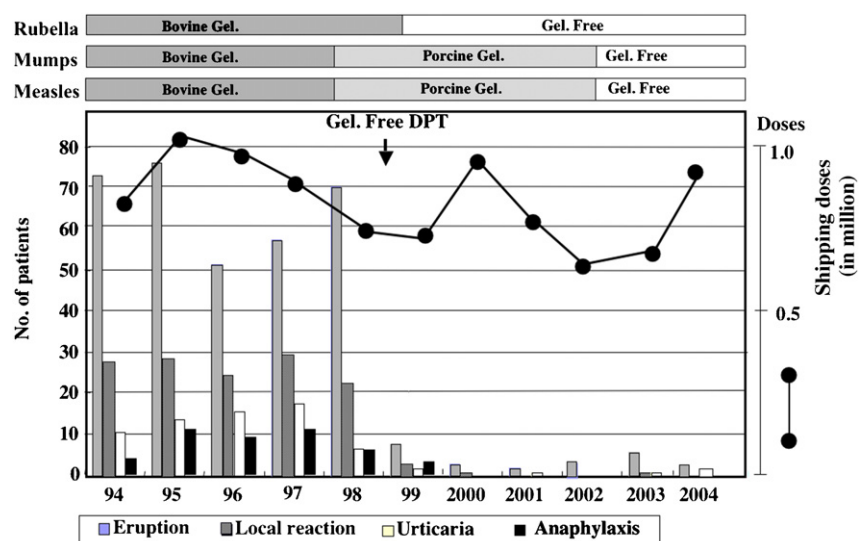


Fig. 2. Annual number of reported cases with allergic reactions, anaphylaxis, urticaria, local reaction and generalized eruption, after vaccination with live measles, mumps and rubella vaccines. The 0.2% bovine gelatin was used in all live vaccines. Gelatin was removed from rubella vaccine in 1999. Porcine gelatin was used in mumps and measles vaccines from 1998 to 2002 and removed from them in 2002. Gelatin was removed from all brands of DTaP in 1999. The dot represents the annual shipping doses of three live vaccines in million.

Table 2
Incidence of allergic reaction after vaccination with inactivated JEV, DPT, and Influenza vaccines, 1994–2004

Allergic reaction	JEV			DPT		Influenza			
	P-M	P-P	G.T. (–)	Gel. (–)	G.T. (–)	P-M	P-P	Gel. (–)	G.T. (–)
Anaphylaxis	2	3	1	9	1	0	4	19	3
Urticaria	3	6	1	6	0	0	2	28	1
Eruption	7	1	1	91	11	5	65	196	9
Local reaction	3	4	1	70	19	4	95	131	14
Shipping (million)	4.78	3.22	1.45	9.68	0.88	0.58	2.12	32.86	2.46
	JEV			DPT		Influenza			
	P-M	P-P	G.T. (–)	Gel. (–)	G.T. (–)	P-M	P-P	Gel. (–)	G.T. (–)
Incidence of anaphylaxis (cases/1 million doses)									
Thimerosal (+)	0.63			0.93		1.48		0.58	
Thimerosal (–)			0.69		1.148				1.22

P-M, porcine gelatin (Merck) 0.02%; Gcl. (–), gelatin free; P-P, porcine gelatin (Prionex) 0.02%; G.T. (–), gelatin, thimerosal free; JEV, Japanese encephalitis virus vaccine.

was reported after vaccination of live vaccines in PMS after 1999 and now, gelatin allergy is settled.

But some anaphylactic reactions were reported after the administration of inactivated vaccines and the results of PMS study for allergic reactions are shown in Table 2. The components of gelatin as a stabilizer and thimerosal as preservatives were modified during the study period of Japanese encephalitis virus vaccine (JEV), DPT and Influenza vaccines. Gelatin has not been used in Kitasato DPT, but the incidence of anaphylaxis is calculated as 0.93 per million immunization practices (nine cases/9.68 million doses). That of DPT free of thimerosal was 1.14 per million doses (one case/0.88 million doses) after 2003. A total incidence of anaphylaxis was 0.95 per million doses of DPT (10 cases/10.56 million doses). We investigated the serum IgE against vaccine components, but we could not identify the causal relationship of the anaphylactic reactions. The incidence of anaphylactic reaction is 0.63 per million for JEV (6 cases/9.45 million doses), and 0.68 for Influenza vaccine (26 cases/38.02 million doses). Most inactivated vaccines have been free of gelatin and thimerosal since 2003, but the incidence of anaphylaxis seemed to be similar, irrespective of the removal of thimerosal.

3.2. Incidence of serious adverse events after vaccination

We summarized the data reported to the PMS Research Unit from April 1994 to December 2004. Table 3 shows the number of reported cases diagnosed as encephalitis and encephalopathy, aseptic meningitis, ADEM, acute cerebellar ataxia, Guillain–Barré syndrome, acute hemiplegia, ITP, and allergic purpura. Shipping doses of measles, rubella, mumps, DPT, JEV, and influenza vaccine are also shown in Table 3.

Among 3.64 million doses of measles vaccine, encephalitis and/or encephalopathy were reported in two patients. One patient with encephalitis had a high fever, measles like eruption, convulsion and loss of consciousness 9 days after vaccination in 2002, and the H1 genotype of the measles virus was detected, which was the circulating genotype in 2002, different from the AIK-C vaccine strain [13]. Among five ITP cases, four bone marrow aspirates were examined and two measles wild-type D5 lineage genomes were detected, circulating strain from 1990 to 1997 [13]. An acute hemiplegia was reported in one case that was diagnosed with congenital intracranial arterial malformation, Moya-moya disease.

Table 3
Number of patients with severe adverse events after vaccination, 1994–2004

	Measles	Rubella	Mumps	DPT	JEV	Influenza
Encephalitis	2 ^a	1	1 ^b	1	1 ^b	0
Aseptic meningitis	0	0	134	0	0	1 ^b
ADEM	0	1	1 ^b	0	2	3
Acute cerebellar ataxia	0	0	1	0	0	1
Guillain–Barré syndrome	0	1	0	0	0	9
Acute hemiplegia	1	0	0	0	0	0
ITF	5 ^a	4	1	0	0	2
Allergic purpura	0	1	1	0	0	0
Toxic shock syndrome	1	0	0	0	0	0
Shipping doses (million)	3.64	4.00	1.53	10.56	9.45	38.02

JEV, Japanese encephalitis virus vaccine.

^a Wild-type measles.

^b Enterovirus infection.

One case each of encephalitis, ADEM and Guillain–Barré syndrome and four cases of ITP were reported among 4.00 million doses of rubella vaccine.

Among 1.53 million doses of mumps vaccine, one case each of encephalitis, ADEM, acute cerebellar ataxia, and ITP and 134 cases of aseptic meningitis were reported. Through virological examinations, the enterovirus genome was detected in the CSF despite being negative for the detection of the mumps virus genome in each patient with encephalitis and ADEM. One hundred and thirty-four patients with aseptic meningitis were reported and we obtained 55 CSF samples from the patients with aseptic meningitis. Mumps virus genome was detected in 40 of them and 35 were identified as the vaccine strain and five as the wild-type.

One patient with encephalitis/encephalopathy was reported out of 10.56 million doses of DPT.

One patient with encephalopathy and two with ADEM were reported among 9.45 million doses of JEV vaccine. The patient was diagnosed as encephalitis caused by enterovirus type 71 infection after a virological examination performed by the Regional Institute for Public Health.

One patient with aseptic meningitis, three with ADEM, nine with Guillain–Barré syndrome, and two with ITP were reported among 38.02 million doses of influenza vaccine. The enterovirus genome was detected in the CSF obtained from the patient with aseptic meningitis.

4. Discussion

The reliability of the surveillance of vaccine adverse events depends on how accurately the surveillance system picks up the cases. The limitation of this study is the self-reporting system by physicians or parents. We realized that the number of mild cases might be underestimated, but the cases with serious illness requiring medical treatment in hospitalization would most likely be registered without missing, because the medical costs for vaccine adverse events are completely compensated under the decision in the advisory committees organized in the regional government or the Ministry of Health, Welfare and Labour by the immunization remedy law, and the cases are verified through legitimate and manufacturer's channels.

Gelatin allergy was first reported after MMR vaccination in the USA [21] and we reported that the anaphylactic reaction was caused by gelatin allergy, with a high prevalence of IgE antibodies against gelatin [6,7]. We speculated that the repetitious administration of DPT containing gelatin enhanced the sensitization against gelatin. After 1999, when even trace amounts of gelatin were removed from all brands of DPT, the incidence of gelatin allergy decreased, along with the modification of gelatin material [20]. No anaphylactic reaction has been reported after immunization with live vaccines in PMS since 1999. In the US, trace amounts of hydrolyzed gelatin are used in DPT and there is 14,500 µg of gelatin in live vac-

cines. The VAERS study group examined 22 subjects with anaphylactic reaction and anti-gelatin IgE was demonstrated in 6(27%). The incidence of anaphylactic reaction was 1.8 per million doses at the time, 1991–1997, and did not increase after the introduction of DTaP containing gelatin [22]. A different incidence of gelatin allergy between Japan and the US was thought to depend on the quality of gelatin used and genetic predisposition [23]. In the case of inactivated vaccines, the incidence of anaphylactic reaction was 0.63 per million for JEV, 0.95 for DPT, and 0.68 for influenza vaccine. Most inactivated vaccines, DPT, JEV, and influenza vaccines have been free of gelatin and thimerosal since 2003, but there was no significant difference in the incidence of anaphylaxis among vaccines with or without gelatin or thimerosal. We could not identify the substances responsible for the allergic reactions, and some patients were reported to have gelatin allergy [24].

Further attenuated vaccines, although extremely less pathogenic, have similar characteristics to their respective wild-types. The wild-type strains have neuro-tropic characteristics by nature [25–27]. Adverse reactions after vaccination were inevitable, and the incidence of encephalitis or encephalopathy was reported as 0.3 cases per 1 million doses of measles vaccine [25]. Measles inclusion body encephalitis (MIBE) and ADEM cases were reported after MMR vaccination, but the causal relationship remains unidentified. One report suggested that the measles virus genome obtained from MIBE after MMR vaccination had a similar sequence to the vaccine strain [28]. Cases of encephalitis after the administration of mumps or rubella vaccines are supposed to be extremely rare events after vaccination.

In a total of 67.2 million immunization practices, 6 cases with encephalitis, 135 aseptic meningitis, 7 ADEM, 10 Guillain–Barré syndrome, 1 hemiplegia, 12 ITP, 2 allergic purpura, and 1 toxic shock syndrome were reported. Two patients with encephalitis were reported after vaccination with measles and the wild-type measles genome was detected from the NPS obtained from one patient. In another case of encephalitis reported after measles immunization, the patient developed febrile reactions with neurological symptoms 3 h after vaccination, and we supposed that it seemed to be a coincidental infection with other pathogens because the events attributed to vaccination occurred between days 6 and 14, at a peak of day 10 after MMR vaccination [29]. MMR vaccine containing the Urabe strain was withdrawn from the market in 1993 because of an unexpectedly high incidence of vaccine-associated aseptic meningitis [30]. The results in this study, using the Hoshino strain, estimated the incidence of aseptic meningitis to be one case in 10,000 vaccine recipients. Among 40 cases where the mumps genome was detected, the vaccine strain was detected in 35, but five were identified as wild strains.

A long-term discussion has been carried out about the causal relationship between vaccines and miscellaneous demyelinating neurological diseases, such as ADEM, Guillain–Barré syndrome, and multiple sclerosis [31,32].

The patho-physiological mechanisms are not fully understood, but possible mechanisms have been proposed; molecular mimicry between vaccine antigen and myelin protein, immunological stimulation by exogenous materials in vaccine components and immunological toxicity of vaccine components. The total estimated incidence of ADEM was 0.10 per million immunization practices and 0.15 per million immunization practices for Guillain–Barré syndrome. Behringwerke reported that the estimated incidence of central nervous system demyelinating illness was 3.5 cases per 10 million within a month of vaccination in 10 years of PMS results [33], similar to our results. The incidence of ITP was 0.18 per million immunization practices.

Enterovirus infection was identified in two patients after mumps vaccination (one each with encephalitis and ADEM), one patient with encephalitis after immunization with JEV vaccine and one with aseptic meningitis after immunization with influenza vaccine. Enterovirus consists of 61 serotypes: coxsackie virus type A (23 serotypes), coxsackie virus type B (6 serotypes), echovirus (28 serotypes), and enterovirus types 68–71 (4 serotypes) [34]. It causes a variety of illnesses, ranging from minor respiratory illness to generalized serious illness, with a short incubation period. In addition, it causes aseptic meningitis, encephalitis, myositis and acute cardiac diseases. Aseptic meningitis is the most common clinical illness caused by enterovirus infection. In Taiwan, epidemics of enterovirus 71 were associated with a high incidence of aseptic meningitis and severe cases with encephalitis had a relatively high case fatality rate in different geographic areas, ranging from 7.7% to 31% [35]. In the present study, one case with encephalitis was diagnosed with enterovirus 71 infection.

In conclusion, several serious vaccine-adverse events have been reported in the PMS study, although the incidence was extremely low, at less than 0.2 per million doses. The coincidental enterovirus infection was identified in some vaccine-adverse events through specific virological laboratory examinations. Thus, we believe that vaccines are safe and that vaccine-adverse events should be continuously monitored with specific laboratory based surveillance to prevent misunderstandings of the causality.

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References

- [1] Expanded programme on immunization. Global advisory group—part I. *Wkly Epidemiol Rec* 1994;69:21–7.
- [2] Chen RT, Rastogi SC, Mullen JR, Hayes SW, Cochi SL, Donlon JA, et al. The vaccine adverse event reporting system (VAERS). *Vaccine* 1994;12:542–50.
- [3] Chen RT, Glasser JW, Rhodes PH, Davis RL, Barlow WE, Thompson RS, et al. Vaccine safety datalink project: new tool for improving vaccine safety monitoring in the United States. *Pediatrics* 1997;99:765–73.
- [4] DeStefano F; vaccine safety datalink project. The vaccine safety datalink project. *Pharmacoepidemiol Drug Saf* 2001;10:403–6.
- [5] Halsey NA. The science of evaluation of adverse events associated with vaccination. *Semin Pediatr Infect Dis* 2002;13:205–14.
- [6] Sakaguchi M, Ogura H, Inouye S. IgE antibody to gelatin in children with immediate-type reactions to measles and mumps vaccines. *J Allergy Clin Immunol* 1995;96:563–5.
- [7] Nakayama T, Aizawa C, Kuno-Sakai H. A clinical analysis of gelatin allergy and determination of its causal relationship to previous administration of gelatin-containing acellular pertussis vaccine combined with diphtheria and tetanus toxoids. *J Allergy Clin Immunol* 1999;103:321–5.
- [8] Hynson JL, Kornberg AJ, Coleman LT, Shield L, Harvey AS, Kean MJ. Clinical and neuroradiologic features of acute disseminated encephalomyelitis in children. *Neurology* 2001;56:1308–12.
- [9] Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain–Barre syndrome. *Ann Neurol* 1990;27(Suppl):S21–4.
- [10] Kobune F, Sakata H, Sugiura A. Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. *J Virol* 1990;64:700–5.
- [11] Plotkin SA. Rubella viruses. In: Lennette EH, Schmidt NJ, editors. *Diagnostic procedures for viral and rickettsial infections*. 4th ed. Washington, DC: American Public Health Association; 1969. p. 364–413.
- [12] Kashiwagi Y, Takami T, Mori T, Nakayama T. Sequence analysis of F, SH, and HN genes among mumps virus strains in Japan. *Arch Virol* 1999;144:593–9.
- [13] Zhou J, Fujino M, Inou Y, Kumada A, Aoki Y, Iwata S, et al. H1 genotype of measles virus was detected in outbreaks in Japan after 2000. *J Med Virol* 2003;70:642–8.
- [14] Yamada A, Takeuchi K, Tanabayashi K, Hishiyama M, Takahashi Y, Sugiura A. Differentiation of the mumps vaccine strains from the wild viruses by the nucleotide sequence of the P gene. *Vaccine* 1990;8:553–7.
- [15] Kashiwagi Y, Kawashima H, Takekuma K, Hoshika A, Mori T, Nakayama T. Detection of mumps virus genome directly from clinical samples and a simple method for genetic differentiation of the Hoshino vaccine strain from wild strains of mumps virus. *J Med Virol* 1997;52:195–9.
- [16] Katow S, Minahara H, Ota T, Fukushima M. Identification of strain-specific nucleotide sequences in E1 and NS4 genes of rubella virus vaccine strains in Japan. *Vaccine* 1997;14:1579–85.
- [17] Takami T, Kawashima H, Takei Y, Miyajima T, Mori T, Nakayama T, et al. Usefulness of nested PCR and sequence analysis in a nosocomial outbreak of neonatal enterovirus infection. *J Clin Virol* 1998;11:67–75.
- [18] Takami T, Sonoda S, Houjyo H, Kawashima H, Takei Y, Miyajima T, et al. Diagnosis of horizontal enterovirus infections in neonates by nested PCR and direct sequence analysis. *J Hospital Infect* 2000;45:283–7.
- [19] Mori T. A simple method for genetic differentiation of the AIK-C vaccine strain from wild strains of measles virus. *Biologicals* 1994;22:179–85.
- [20] Nakayama T, Aizawa C. Change in gelatin content of vaccines associated with reduction in reports of allergic reactions. *J Allergy Clin Immunol* 2000;106:591–2.
- [21] Kelso JM, Jones RT, Yunginger JW. Anaphylaxis to measles, mumps, and rubella vaccine mediated by IgE to gelatin. *J Allergy Clin Immunol* 1993;91:867–72.
- [22] Pool V, Braun MM, Kelso JM, Mootrey G, Chen RT, Yunginger JW, et al. Prevalence of anti-gelatin IgE antibodies in people with anaphylaxis

- after measles–mumps–rubella vaccine in the United States. *Pediatrics* 2002;110.6:e71.
- [23] Kumagai T, Yamanaka T, Wataya Y, Saito A, Okui T, Yano S, et al. A strong association between HLA-DR9 and gelatin allergy in the Japanese population. *Vaccine* 2001;19:3273–6.
- [24] Sakaguchi M, Miyazawa H, Inouye S. Specific IgE and IgG to gelatin in children systemic cutaneous reactions to Japanese encephalitis vaccines. *Allergy* 2001;56:536–9.
- [25] Strebel PM, Papania MJ, Halsey NA. Measles vaccine. In: Plotkin SA, Orenstein WA, editors. *Vaccines*. 4th ed. Philadelphia: Saunders; 2004. p. 389–440.
- [26] Plotkin SA. Mumps vaccine. In: Plotkin SA, Orenstein WA, editors. *Vaccines*. 4th ed. Philadelphia: Saunders; 2004. p. 441–69.
- [27] Plotkin SA, Reef S. Rubella vaccine. In: Plotkin SA, Orenstein WA, editors. *Vaccines*. 4th ed. Philadelphia: Saunders; 2004. p. 707–43.
- [28] Bitnun A, Shannon P, Durward A, Rota PA, Bellini WJ, Graham C, et al. Measles inclusion-body encephalitis caused by the vaccine strain of measles virus. *Clin Infect Dis* 1999;29:855–61.
- [29] Virtanen M, Peltola H, Paunio M, Heinonen OP. Day-to-day reactivity and the healthy vaccinee effect of measles–mumps–rubella vaccination. *Pediatrics* 2000;106:e62.
- [30] Ueda K, Miyazaki C, Hidaka Y, Okada K, Kusuha K, Kadota R. Aseptic meningitis caused by measles–mumps–rubella vaccine in Japan. *Lancet* 1995;353:2026–9.
- [31] Stratton KR, Howe CJ, Johnston JB. Adverse events associated with childhood vaccines other than pertussis and rubella. *JAMA* 1994;271:1602–5.
- [32] Gout O. Vaccinations and multiple sclerosis. *Neurol Sci* 2001;22:151–4.
- [33] Quast U, Herder C, Zwisler O. Vaccination of patients with encephalomyelitis disseminata. *Vaccine* 1991;9:228–30.
- [34] Pallansch M, Roos R. Enteroviruses: polioviruses, coxsackie-viruses, echoviruses, and newer enteroviruses. In: *Fields virology*. 4th ed. New York, NY: Lippincott-Raven; 2001. pp. 1267–312.
- [35] Ho M, Chen ER, Hsu KH, Twu SJ, Chen KT, Tsai SF, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N Engl J Med* 1999;341:929–35.