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Tetsuo Nakayama, Shigeru Suga, Kenji Okada, and Nobuhiko Okabe

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Tetsuo Nakayama 1), Shigeru Suga 2), Kenji Okada 3), Nobuhiko Okabe 4)

1) Kitasato Institute for Life Sciences, Laboratory of Viral Infection, Tokyo 108-8641, Japan
2) National Mie Hospital, Department of Pediatrics, Mie 514-0125, Japan
3) Fukuoka Nursing College, Fukuoka 814-0193, Japan
4) Kawasaki City Institute for Public Health, Kawasaki 210-0821, Japan

Corresponding author: T. Nakayama,
Kitasato Institute for Life Sciences,
Department of Viral Infection
Shirokane 5-9-1, Minato-ku, Tokyo 108-8641
TEL: 81-3-5791-6269, FAX: 81-3-5791-6130, E-mail: tetsuo-n@lisci.kitasato-u.ac.jp

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著者

1）中山哲夫：北里生命科学研究所 ウイルス感染制御 II

2）岡田賢司：福岡看護大学 基礎・基礎看護部門

3）菅 秀：国立病院機構 三重病院 小児科

4）岡部信彦：川崎健康安全研究所
SUMMARY: A follow-up serological study was conducted involving 47 subjects who received four doses of diphtheria and tetanus toxoids combined with acellular pertussis vaccine (DTaP) together with Salk-type inactivated polio vaccine (DTaP-wIPV) until 6 years of age. All antibody levels declined more rapidly than expected within 3 years after the completion of the primary vaccination with 4th booster dose and titers persisted until 6 years of age. The positive rate of the IgG antibody against pertussis toxin (PT) was 31.9% (15/47) at 4 years of age, 41.0% (16/39) at 5 years of age, and 40.5% (15/37) at 6 years of age. A significant increase in the PT antibody was observed in 6 subjects, suggesting subclinical infection. Positive rates of antibodies against other targets did not decrease but titers of neutralizing antibodies against poliovirus type III decreased in only a few subjects. These data suggest the need for an additional preschool booster immunization using DTaP-wIPV.
INTRODUCTION

Poliomyelitis is an infectious disease caused by poliovirus types I, II, and III. It infects through the alimentary tracts and causes viremia leading to infection of the anterior horn of the cerebrospinal cord, resulting in serious neurological complications with acute flaccid paralysis. A nationwide outbreak was reported in 1960-61 in Japan, and the emergent administration of oral polio vaccine (OPV) was implemented in 1961. After the introduction of OPV to the recommended immunization, the number of reported cases of paralytic poliomyelitis reduced rapidly on applying a two-dose schedule of OPV, and no case of polio paralysis caused by circulating wild virus has been reported since 1981 (1). OPV is an attenuated virus, but extremely rare vaccine-associated paralytic poliomyelitis (VAPP) can occur, affecting one in a few million vaccine recipients caused by back-mutation of the attenuated strain (2, 3). In Japan, all reported cases of paralytic poliomyelitis have been due to VAPP since 1981. In most developed countries, OPV was replaced by inactivated polio vaccine (IPV) to avoid the risk of VAPP, preventing infection with wild-type polio virus.

IPV (IMOVAX POLIO®, Sanofi) was licensed in April 2012, and introduced in the market for routine immunization in September 2012. Diphtheria and tetanus toxoids combined with acellular pertussis vaccine (DTaP) together with IPV from live Sabin strains (DTaP-sIPV) was licensed by two manufacturers in Japan (4). Polyvalent combined vaccines are currently used throughout the world and DTaP-wIPV has been a basic vaccine (5,6). DTaP-wIPV from Kitasato Daiichi Sankyo Vaccine was licensed in July 2014 and introduced in the market for routine immunization in December 2015.

Recently, the resurgence of pertussis has become a critical issue worldwide, especially among young adolescents and adults due to the gradual loss of vaccine-acquired immunity against pertussis (7, 8, 9). The persistence of antibodies against pertussis is a matter of concern for vaccine effectiveness. From meta-
analysis regarding the period of pertussis immunity following DTaP vaccination, the waning immunity was evident, and an estimated average duration of vaccine protection was less than three years after the last dose of DTaP vaccination (10). In Japan, with a long history of DTaP, the number of reported cases of pertussis has increased in young adolescents and adults since 2002 (11, 12). However, recently, the peak age-specific incidence was 7-9 years (13).

In most countries, three doses are used as primary immunization at 2, 4, and 6 months of age with a booster dose at one year after the third dose with additional booster immunization at 4-6 years of age, and multivalent combined vaccines based on DTaP-wIPV have been introduced in most developed countries (5, 6). However, no additional dose at 4-6 years is scheduled in Japan. In the present study, we examined the antibodies against each component of DTaP-wIPV following immunization to investigate the persistence of antibodies and discussed the appropriate timing for an additional dose.

**MATERIALS AND METHODS**

**Study design and subjects:** A phase III clinical trial of DTaP-wIPV (DD687-A-J301) was conducted during 2011-2012 (14). As the participants of the DD687-A-J301 clinical trial, 47 subjects in 10 pediatric clinics or general hospitals were enrolled in 2015 (4 years of age) in the present study, 39 subjects in 2016 (5 years of age) and 37 subjects in 2017 (6 years of age), whose guardians consented to their participation. They received the fourth dose of DTaP-wIPV at one year of age in 2012, and clinical study was conducted from 2015 to 2017. The study design was approved by the ethics committee of National Mie Hospital (chief investigator: Dr. Shigeru Suga). The study was organized by the Working Group of the Japanese Society for Vaccinology.
**Serological study:** Serum samples were obtained at 4, 5, and 6 years of age, and antibodies against the pertussis toxin (PT) and filamentous hemagglutinin (FHA) using the DENKA SEIKEN pertussis EIA kit (DENKA SEIKEN, Tokyo, Japan), antibody against diphtheria toxin using the Park-Williams No. 8 strain, KPA agglutination antibodies for tetanus toxoid of the Harvard strain, and neutralizing antibodies against wild-type poliovirus I (Mahoney strain), poliovirus II (MEF-1 strain), and poliovirus III (Saukett strain) were examined. Antibodies against DTaP were measured in KITASATO-OTSUKA Biomedical Assay Laboratories Co. Ltd., and neutralizing antibodies against polioviruses I, II, and III were assayed in Sanofi Pasteur Global Clinical Immunogenicity Laboratories (15).

Cut-off levels were defined as >10 EU/mL for PT and FHA IgG antibodies, 0.1 IU/mL for diphtheria antitoxin, 0.01 IU/mL for tetanus antitoxin, and ≥8 neutralizing antibody titers against polioviruses are considered protective levels against paralytic poliomyelitis.

Serum antibody data from the clinical trial were used for the serum titers at one year of age one month after the booster dose. Antibody assay was performed with the same procedures in the clinical trial and present study.

**RESULTS**

**Antibody against DTaP:** The results of the five-year follow-up serological study of PT antibodies are shown in Fig. 1a. All subjects were seropositive for PT antibody immediately after the completion of the 4th booster doses, but the positivity of PT antibody was 31.9% (15/47) at 4 years of age, 41.0% (16/39) at 5 years of age, and 40.5% (15/37) at 6 years of age. A significant increase in PT antibody was observed in 4 out of 39 subjects at 4-5 years of age, and 2 of 37 subjects at 5-6 years of age. The results of serological
examination of FHA are shown in Fig. 1b. All subjects were seropositive for FHA antibody after the 4th booster dose and the positivity reduced to 76.6% (36/47) at 4 years, 92.3% (36/39) at 5 years of age, and 89.2% (33/37) at 6 years of age. A significant increase in FHA antibody was observed in 7 of 39 subjects at 4-5 years of age, and 6 of 37 subjects at 5-6 years of age (Fig. 1b).

The results of antibody levels against diphtheria and tetanus toxins are shown in Fig. 2a and b. The 100% positivity of the antibodies against diphtheria and tetanus toxins was maintained. The antibody against diphtheria and tetanus toxins decreased at 4 years of age and was maintained at similar levels until 6 years of age.

**Antibody against poliovirus types I, II, and III:** The results of the follow-up study of neutralizing antibodies against poliovirus types I, II, and III are shown in Fig. 3. Immediately after the 4th booster dose, high neutralizing antibody levels >1,000 were observed and declined at 4 years of age, but antibody levels were maintained until 6 years of age. The positive rate of neutralizing antibody against poliovirus type III decreased to 93.6% (44/47) at 4 years of age, 94.9% (37/39) at 5 years of age, and 94.6% (35/37) at 6 years of age.

**DISCUSSION**

All antibodies decreased at 4 years of age three years after the last dose of primary immunization and showed similar levels until 6 years of age. The decline of PT antibody levels was remarkable, the positive rate decreased to 31.9% (15/47) at 4 years of age, and significant increases in PT and FHA antibodies were observed in several subjects. They did not demonstrate typical symptoms, and we suspected asymptomatic infection because regional sporadic outbreaks were reported during the study periods. McGirr et al. (10)
reported that the annual odds of pertussis for every additional year after the last dose of DTaP increased by 1.33 times (95% confidence interval: 1.23-1.43) from a meta-analysis, showing no significant difference for the 3- versus 5 dose DTaP regimens. They recommended that earlier and repeated booster strategies are necessitated to achieve herd immunity to control the spread of pertussis. Gustafson et al. (16) reported that the incidence of pertussis was lowest at 6 years of age but increased at 7 and 8 years of age and suggested the need for an additional dose of DTaP at 5-7 years of age. Zepp et al. (17) reported that a preschool booster reduced the total number of patients with pertussis in all generations, not only for the targeted school-aged children, because of indirect effects. In Norway, PT antibody was investigated at 6-12 years of age, and the results indicated that the level of immunity against pertussis was low 5 years after primary vaccination and that the DTaP-booster administered at 7–8 years generated a moderate anti-pertussis immune response that waned to near pre-booster levels in a few years (18). Although a pre-school dose is administered at age 4 years in the U.S., the highest incidence was in children aged 7-14 years, peaking at 10 years of age (9).

In our previous study, the prevalence of PT antibodies was investigated at three points: the 1st grade of elementary school (6-7 years of age), junior high school (12-13 years), and university students (18-20 years) during 2013-2015 (19). The positive rate of PT antibodies was less than 50% at the 1st grade of elementary school and increased to 60% at 12-13 years of age, and to 73% at 18-19 years of age in 2013-2014. The mean PT antibody titer increased from 16-21 EU/mL at 6-7 years to 22-26 EU/mL at 12-13 years 23-36 EU/mL at 18-19 years without demonstrating pertussis-like illness. Besides this cross-sectional serological study, the peak age-specific incidence of pertussis was 7-12 years, confirmed by the detection of pertussis genome in the epidemiological study (13). Therefore, an additional dose of DTaP should be added to the routine immunization schedule.
In most developed countries, the preschool booster was administered using polyvalent vaccines based on DTaP-wIPV. In Japan, DTaP-sIPV was licensed and the persistence of antibodies against poliovirus is of interest (20). In the present study, 47 subjects in the phase III trial of DTaP-wIPV were enrolled and the duration of the persistence of neutralizing antibodies was assessed against polioviruses type I, II, and III. High protective antibody levels were maintained in children until 6 years old, but antibody levels against poliovirus type III decreased in a few subjects. The persistence of antibody against polioviruses was assessed globally through long experience of DTaP-wIPV and pentavalent or hexavalent vaccines (DTaP-wIPV combined with *haemophilus influenzae* type B vaccine and/or hepatitis B virus vaccine (21, 22, 23). Regarding the necessity of a preschool booster for polio vaccine, long-term protection should be considered beyond just school-aged generation, possibly in adolescent.

In conclusion, all antibody levels declined more rapidly than expected within 3 years after the completion of the 4th booster dose and protective antibody levels persisted until 6 years of age. However, PT antibodies decreased in a large proportion and subclinical infections were suspected through present serological study with a significant increase in PT antibody, together with our previous epidemiological report (13). Positive rates of antibodies against other targets did not decrease but titers of antibodies against poliovirus type III decreased in only a few subjects. These data suggest the need for an additional preschool booster immunization using DTaP-wIPV.
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Authors’ contributions

The corresponding author TN summarized the data and prepared the manuscript. Dr. KO contributed to design the protocol and performed the study. Dr. SS and NO organized study.
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**Figure legends**

Fig. 1. Duration of persistence of antibodies against PT IgG and FHA IgG EIA antibodies of *Bordetella pertussis*.

Antibody titers at 1 year were from the results of a phase III clinical trial. Serum samples were obtained at 4, 5, and 6 years of age.

Fig. 2. Duration of persistence of antibody against diphtheria and tetanus toxoids.

Antibody titers at 1 year were from the results of a phase III clinical trial. Serum samples were obtained at 4, 5, and 6 years of age.

Fig. 3. Duration of persistence of neutralizing antibody titers against poliovirus types I, II, and III.

Neutralizing antibody titers at 1 year were from the results of a phase III clinical trial. Serum samples were obtained at 4, 5, and 6 years of age.
Fig. 1

(a) PT IgG antibody

(b) FHA IgG antibody

1 year 4 years 5 years 6 years

1 10 100 1000 10000

EU/mL
Fig. 3

(a) Neutralizing antibody against poliovirus type I
(b) Neutralizing antibody against poliovirus type II
(c) Neutralizing antibody against poliovirus type III