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Note

Vaccine acquired pertussis immunity was weakened at 4 years of age and asymptomatic pertussis infection was suspected based on serological surveillance☆

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ABSTRACT

Serological surveillance of pertussis antibodies was performed in 118 children aged 1–12 years. The positivity of pertussis toxin (PT) antibodies was low at 4–6 years and significantly higher at 8–9 years, compared with those at 6 years. Fimbriae 2 (Fim2) antibody showed similar response to the PT antibody. Higher antibody titers against Fim3 were observed among subjects ≥5 years and highest at 8 years. Data demonstrated that the vaccine-induced antibodies decayed by 4–5 years and subclinical pertussis infection was suspected thereafter, suggesting the need for additional dose at around 4–5 years.

Whole-cell inactivated pertussis vaccine was introduced into immunization program in 1950, and diphtheria toxoid, tetanus toxoid and whole-cell pertussis (DTwP) vaccine was recommended for routine immunization schedule in 1968 in Japan. The number of patients with pertussis decreased after introduction of the vaccine. However, it caused accidental deaths following immunization with DTwP in 1974-75 [1]. DTwP was suspended in 1975 and diphtheria and tetanus toxoids and acellular pertussis (DTaP) was introduced in 1981 [1]. Two types of DTaP composed different purified components of *Bordetella pertussis* (*B. pertussis*) have been available: one with pertussis toxin (PT) and filamentous hemagglutinin (FHA) (DTaP2), and another with additional pertactin and fimbriae 2 (Fim2) (termed polyvalent DTaP). Since 1981, the number of reported cases of pertussis has decreased due to the acceptance of DTaP [1], but patients with pertussis were increasingly reported in the late 1990's [2,3]. In Japan 2010-11, several regional outbreaks were reported in young adolescents and adults, and recently the number of patients with pertussis shifted to school-age children [4].

In the U.S. and E.U., diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus (DTaP-IPV) vaccines are administered three times before one year of age, a booster dose one year after the last of the initial three doses, and additional dose at 4–6 years [5]. However, one additional dose at 4–6 years is not scheduled in Japan [1,5]. In our previous study, positivity of PT antibodies was less than 50% at the 1st grade of elementary school and increased at the first grade of junior high school and university with increasing mean titers [6].

During the periods 2011–2012 and 2013–2014, sero-epidemiological investigation against respiratory infections was conducted. A total of 118 serum samples were obtained from healthy children from one year to 12-years-old in 2012. All subjects were immunized with scheduled doses of DTaP. The study protocol was approved by the Ethics Committee of Yamaguchi

Abbreviations: B. pertussis, *Bordetella pertussis*; DTaP, diphtheria and tetanus toxoids and acellular pertussis; DTaP-IPV, diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus; DTwP, diphtheria toxoid, tetanus toxoid and whole-cell pertussis; EIA, enzyme-linked immunoassay; EU, Japanese EIA units; FHA, filamentous hemagglutinin; Fim, Fimbriae; PT, pertussis toxin.

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University, and informed consent was obtained from the parents. IgG antibodies against PT and FHA were assayed using the enzyme-linked immunoassay (EIA) kit (Denka Seiken, Tokyo, Japan). Positivity for PT and FHA IgG antibodies was defined as ≥10 Japanese EIA units (EU). IgG antibodies against Fim2 and Fim3 were examined by fluorescent immunosorbent assay modified from the conventional EIA method [7]. EIA plate wells were coated with 20 ng of Fim2 and 22 ng of Fim3. Serum samples were diluted at 1:100 in 1% BSA-PBS and applied in antigen-coated wells for 1 h. Anti-human IgG antibody raised in a goat diluted at 1:4,000, labeled with β-galactosidase (American Qulex Antibodies, CA, USA) was added, and 4 MU β-galactoside (Sigma-Aldrich, Inc., MO, USA) was added for the substrate to emit fluorescence. The fluoro-units (FU) was measured using Fluoroscan II (Labsystems, Ontario, Canada) [8]. Cut-off level was considered as 500 FU from the results of follow-up study of 35 nursing staff members for 3.5 years, having a contact with a patient with pertussis [8]. The results are shown in box-and-whisker plot with median titer, lower and upper quartile and whisker rang of 5–95%. Statistical significance was evaluated by Mann-Whitney *U* test.

The results of PT and FHA antibodies are shown in Fig. 1. The positive rates and mean titers of PT antibodies were low at 4–6 years of age and showed significantly higher levels at 8–9 years, compared with those at 6 years. PT > 100 EU suggests the possibility of recent infection [9], and the arrow of the outlier at 9 years shows a subject with high PT antibody titers of 111.5 EU (upper panel). FHA antibody was lowest at 4 years and showed higher at 7 and 9 years ($p < 0.05$).

Current circulating isolates express fimbriae 3 (Fim3), and otherwise the Tohama vaccine strain expresses Fim2 [10]. Fim3 is not included in Japanese DTaP vaccines. The results of Fim2 antibodies are shown in the upper panel of Fig. 2. Fim2 antibodies at one year of age were divided into two groups; among 8 subjects, 5 showed around 2000 FU and remaining 3 showed <500 FU. In Japan at that time, four brands of DTaP were available. Two brands of DTaP vaccines contained two components of PT and FHA and the other two contained additional pertactin and Fim2 antigens. Unfortunately, no immunization history of DTaP brands was obtained but subjects with high titers against Fim2 were supposed to be immunized with polyvalent DTaP. The mean Fim2 titer was lowest at 4 years in comparison with that at one year and showed significantly higher levels at 5 years in comparison with that at 4 years. The levels of Fim2 antibody titers showed low levels at 8–10 years.

The results of Fim3 antibodies are shown in the lower panel of Fig. 2. The cut-off level was >500 FU. There was no significant change until 4 years, but few serum samples showed high titers. They are supposed to be infected with *B. pertussis*. Fim3 antibody levels at 5, 6, 7, and 9 years showed a significant higher level ($p < 0.05$) and the highest at 8 years (Median 901 FU, 95% CI: 709-1,164 FU, $p < 0.01$) compared with those at 1 year. Subjects in the present study had no clinical history of pertussis infection. A serum sample with a high PT antibody of 111.5 EU at 9 years (shown in red arrow of outlier in the upper panel of Fig. 1) showed 1,479 FU of Fim3 antibody and 246 FU of Fim2 antibody.

The diagnosis of pertussis is based on bacterial isolation, genome detection, and serological examination of PT antibodies with difficulties of the persistence of vaccine-acquired immunity [11]. Subjects with a high PT antibody titer >100 EU are suspected to have been recently infected, but high levels of PT antibody were found to persist for a long time after pertussis infection. High levels of PT antibody do not always indicate recent infection with pertussis, but strongly suggest the infection [9,11].

Detection of Fim2 and Fim3 antibodies can discriminate between natural infection and vaccine immunity and, therefore, high titers of anti-Fim3 antibodies indicate recent pertussis infection [8]. High levels of Fim3 antibodies were observed in serum samples obtained from the patients with recent pertussis infection, being consistent with the predominant serotype of isolates in the U.K. Despite being circulating Fim3 serotypes, moderate increase in Fim2 antibodies were observed, probably because of cross reaction between Fim2 and Fim3 depending on the previous immunization history of vaccine brands containing of Fim2 antigen [12]. Recent reports showed an equal likelihood of exposure to Fim2 and Fim3 antigens, despite most isolates being of the Fim3 only [13,14].

In the present study, PT antibody was low at 4 years and showed high titers at 8–9 years. Anti-Fim2 antibody decreased at 4 years,

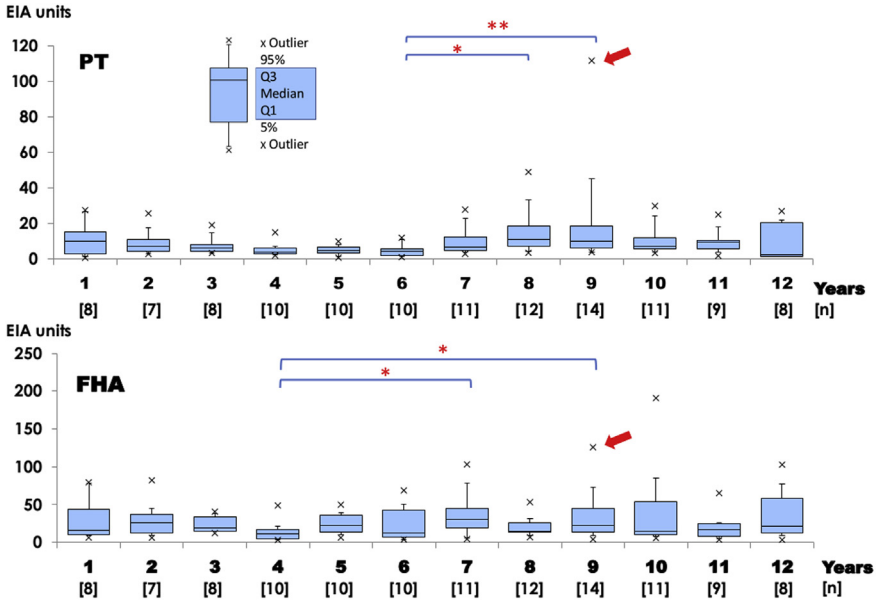

Fig. 1. PT and FHA antibodies in box-and-whisker plot with median titer with lower and upper quartile and whisker rang of 5–95%. The red arrow shows the PT antibody titer >100 EU. Statistical significance was shown (*: $p < 0.05$, **: $p < 0.01$).

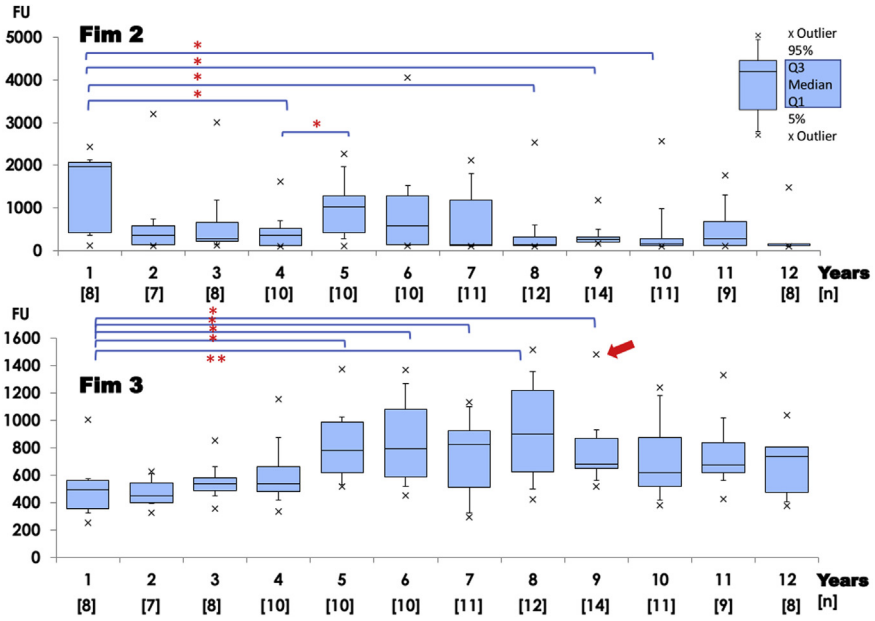

Fig. 2. Anti-Fim2 and anti-Fim3 antibodies in box-and-whisker plot with median titer with lower and upper quartile and whisker rang of 5–95%. The Red arrow indicates Fim2 and Fim3 antibody titers of the serum sample showing PT antibody titer >100 EU. Statistical significance was shown (*: $p < 0.05$, **: $p < 0.01$).

suggesting the decay of vaccine-acquired immunity. Anti-Fim3 antibody showed significant higher levels at 5–9 years in comparison with that of 1 year. These results indicate the possibility of asymptomatic infection of pertussis and the need for an additional dose of DTaP before entering of primary school.

Conflicts of interest

The corresponding author Nakayama T. has received research funding from Daiichi Sankyo Pharmaceutical. Noda A. is an employee of Kitasato Bio-Medical Assay Research Institute.

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