Acellular Pertussis Vaccines Developed in Japan and Their Application for Disease Control

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Acellular pertussis vaccines, comprising mainly pertussis toxin and filamentous hemagglutinin (FHA) and used successfully in Japan since 1981, were evaluated. Anti-pertussis toxin antibody responses in children immunized with acellular pertussis vaccines were comparable or higher than in convalescent-stage patients with pertussis, while those for anti-FHA were far higher. Reactogenicity was much less than with whole cell pertussis vaccines: Fever $\geq38^\circ C$ occurred in 2%-4% of vaccinees; there was little redness $\geq5$ cm in diameter at the vaccination site with the first dose but occurred in 7%-8% of vaccinees with subsequent doses. The estimated efficacy of acellular pertussis vaccine in children ages 2-8 years was 84% (95% confidence interval, 71%-91%). Since vaccine-induced immunity weakens after 6-10 years, adults are now a major source of pertussis transmission. The source of infection was an adult in 11.2% (10/89) of cases, and the secondary attack rate was 10.3% (19/185). Immunization with acellular pertussis vaccines for both adults and children is recommended for disease control.

There is no doubt about the efficacy of diphtheria–tetanus toxoid–whole cell pertussis (DTPw) vaccine in Japan; however, 2 fatalities occurred among children vaccinated with the same lot of DTPw in 1974 and 1975. The Japanese Ministry of Health and Welfare halted DTPw vaccination immediately after the 1975 death. DTPw vaccination was restarted 2 months later, but the participation rate dropped from 77.8% in 1974 to 22.1% in 1975 and 13.6% in 1976. Despite a rise in the vaccination rate to 41.5% in 1977 and 64.4% in 1978, the incidence of pertussis increased markedly during this period (figure 1).

The infection caused by *Bordetella pertussis* is believed to have two stages, respiratory colonization and toxin-mediated disease, with various toxins and virulent factors of *B. pertussis* involved in these stages. It is now clear that the protective antigens against pertussis infection are pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (a 69-kDa outer membrane protein [OMP]), and fimbriae (aggulutinogens). However, because of the demand for a new vaccine with minimal adverse reactions, the use of the Japanese DT–acellular pertussis (DTPa) vaccine became widespread before the pertussis antigens in the vaccine were completely characterized. At the time of its introduction, the only efficacy requirement for DTPa was its potency, determined using the mouse intracerebral protection test [1]. With the advent of improved techniques for antigen characterization, it has become clear that the antigenic constituents of the Japanese DTPas vary by manufacturer, although all contain at least PT and FHA antigens [2].

The Vaccination Research Group of Keio University (Diph group) has played a leading role in the introduction and assessment of DTPa. Clinical trials with the vaccine and research into pertussis by the group have evaluated adverse reactions and antibody responses [3], clinical vaccine efficacy, and pertussis in adults.

**Adverse Reactions and Antibody Responses to DTPa Vaccine**

To assess safety and antibody responses to DTPa, 350 healthy children ages 3 months to 2 years who were based in a special residential facility were enrolled in the study. Subjects received three primary doses of DTPa at monthly intervals and a booster dose 1 year later. Each dose had 0.5 mL of vaccine and was administered by deep subcutaneous injection in the deltoid region.

Two batches of commercially available DTPa from each of three manufacturers were used (table 1). The DTPas made by Biken and Takeda (both Osaka, Japan) were licensed by the US Food and Drug Administration and are now available for use as a booster dose in the United States. Biken batches 11 and 17 contained 5–6 μg of protein nitrogen (PN)/mL PT and FHA but not pertactin or fimbriae. Takeda lots 15 and 20 contained 1.3–1.5 μg of PN/mL PT and 10–12 μg of PN/mL FHA and also contained both pertactin and fimbriae. Kitasato (Tokyo) lots 17 and 20 contained 1.4–2.0 μg of PN/mL PT and 10–12 μg of PN/mL FHA. Their pertactin and fimbriae contents were not tested. All of the DTPa vaccines tested in this study contained aluminum as an adjuvant.

**Adverse reactions.** Adverse reactions to the DTPa vaccines were evaluated by examining patients for systemic fever, local reactions at the injection site, and serious systemic reactions. The incidence of fever $\geq38^\circ C$ within 48 h of DTP immunization was 2.5% (7/274) for Biken vaccines, 4.5% (14/316) for...
forearm swelling or blisters in 1 patient in every few hundred DTPa injections.

Although no serious systemic reactions were observed, severe adverse reactions to DTPa cannot be adequately assessed in clinical follow-up studies because the incidence is so low. However, data on severe adverse reactions are available by analyzing claims submitted to the national compensation system for injuries allegedly resulting from mandatory vaccination [4] (table 2). From 1975–1980, when DTPw was used, of 19.8 million doses, there were 8 severe adverse reactions with sequelae, including 3 deaths, a rate of 0.411/10^6 doses. Between 1981 and 1984, when DTPa was in use, the rate decreased to 5 cases and 2 deaths in 20.4 million doses (0.25 severe reactions/10^6 doses). Although no official data on the incidence of severe reactions from 1985 to the present are available, the incidence appears to be lower than during 1981–1984. Compensation is usually awarded to patients with sequelae considered possibly caused by pertussis vaccine unless proven otherwise. It is difficult to exclude the vaccine as a causal factor, even when other etiologies are suspected, particularly when the adverse events occur in close temporal proximity to vaccination. Overall, there are few severe adverse reactions with DTPa.

Antibody responses. Serologic studies were done for 149 children enrolled in the study. Blood was drawn by venipuncture before the first immunization and before the booster. Blood was also obtained 4 weeks after the last primary series immunization and the booster. Anti-PT and anti-FHA antibody titers were measured using a glass bead ELISA developed in our laboratory [5]. Agglutinin titer was measured by the microagglutination test using the serotype 1.3 strain; in Japan, 90% of pertussis strains isolated are of this serotype [6].

Satisfactory serum IgG antibody responses to PT and FHA were observed after three primary doses and a booster dose of the Biken vaccine [5]. The patterns of antibody changes observed after the other five lots of acellular vaccines were similar.

In patients with pertussis, anti-PT and anti-FHA antibodies reach peak levels 6–10 weeks after disease onset. Therefore, antibody levels in vaccinees 4 weeks after the primary vaccination series were compared with those of pertussis patients 6–

### Table 1. Content of diphtheria–tetanus toxoid–acellular pertussis vaccines by manufacturer.

<table>
<thead>
<tr>
<th>Manufacturer, batch no.</th>
<th>Pertussis antigen (µg of PN/mL)</th>
<th>PT (µg of PN/mL)</th>
<th>FHA (µg of PN/mL)</th>
<th>Fimbriae (µg of PN/mL)</th>
<th>Other (µg of PN/mL)</th>
<th>Aluminum (mg/mL)</th>
<th>Diphtheria (Lf/mL)</th>
<th>Tetanus (Lf/mL)</th>
</tr>
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<tbody>
<tr>
<td>Biken, 11</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>--</td>
<td>--</td>
<td>0.2</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>17</td>
<td>10.0</td>
<td>5.0</td>
<td>5.0</td>
<td>--</td>
<td>--</td>
<td>0.15</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>Takeda, 15</td>
<td>14.6</td>
<td>1.5</td>
<td>12.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>25</td>
<td>3.5</td>
</tr>
<tr>
<td>20</td>
<td>12.1</td>
<td>1.3</td>
<td>10.3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>25</td>
<td>3.5</td>
</tr>
<tr>
<td>Kitasato, 17</td>
<td>14.0</td>
<td>1.4</td>
<td>11.9</td>
<td>NT</td>
<td>0.7</td>
<td>0.15</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>14.0</td>
<td>2.0</td>
<td>10.0</td>
<td>NT</td>
<td>2.0</td>
<td>0.15</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

NOTE. Data are from manufacturers and were reported in [3]. PN, protein nitrogen; PT, pertussis toxin; FHA, filamentous hemagglutinin; NT, not tested.
The immunogenicity of DTPa in children of different ages was also investigated. Anti-PT and anti-FHA antibody responses were compared 4 weeks after the third dose of DTPa (Kitasato batch 17), according to the child’s age when the first dose was given. The immunogenicity of this DTPa vaccine in children ages 3–6 months and 2 years did not differ [3] (figure 3).

Clinical Efficacy of DTPa and DTPw Vaccines

Protection of households [3, 7]. Since our knowledge of the protective immune responses against *B. pertussis* is incomplete, proof of protection against the disease is at present the only acceptable measure of vaccine efficacy. Therefore, we assessed the protective efficacy of DTPa by comparing the secondary attack rates in household contacts of children immunized with DTPa, DTPw, or both and those not immunized. In total, 546 patients with pertussis who attended Keio University Hospital and its affiliated hospitals in the Tokyo metropolitan area from 1981 to the present were entered into the study. Of these 546 index cases, 247 (45.2%) were confirmed by culture. The household contacts of each patient were surveyed in the clinic or by telephone, and symptoms, vaccination status of all children in the household, and the occurrence of any primary or secondary cases were ascertained. This survey identified 557 children who were household contacts. In fully immunized children (≥3 doses of DTP) ages 2–8 years, the secondary attack rate was 12 (9.6%) of 125 after DTPa injection, 7 (13.5%) of 52 after DTPw, and 5 (13.5%) of 37 in those who received both vaccines. The attack rates in children immunized with DTPw and DTPa did not differ. In unimmunized children ages 2–8 years, the attack rate was 50 (58.8%) of 85, which was higher at a 1% significance level, than that in any fully immunized group. The estimated efficacy of DTPa in children ages 2–8 years was 84% (95% confidence interval [CI], 71%–91%; table 3).

Because the content of DTPa varies by manufacturer, the secondary attack rates for the vaccines were calculated by manufacturer as follows: 1 of 8 for Biken, 3 of 38 for Takeda, and 1 of 17 for Kitasato. The number of children fully immunized with each vaccine was small, so vaccine efficacies could not be calculated. However, DTPa produced by all three manufacturers appeared to be similarly effective.

Protection of young infants [8]. Data on vaccine efficacy in children ≤1 year old could not be determined in the household contact study. Fortunately, we were able to conduct a prospective study of a pertussis outbreak in a residential facility housing 19 children ≤2 years old. Ten of the children had not been immunized and 9 had been immunized with DTPa. Of the 10 nonimmunized children, 7 acquired laboratory-confirmed pertussis (4-fold titer rise, positive culture, or both) and 6 of the

### Table 2. Severe adverse reactions after pertussis vaccination.

<table>
<thead>
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<tr>
<td>Severe reactions with sequelae (deaths)</td>
<td>19.8</td>
<td>20.4</td>
</tr>
<tr>
<td>Incidence/10^6 doses (deaths)</td>
<td>0.4 (0.15)</td>
<td>0.25 (0.10)</td>
</tr>
</tbody>
</table>

NOTE: Data are from [4].
Figure 3. Antibody responses to pertussis toxin (left) and filamentous hemagglutinin (right) 4 weeks after 3 doses of acellular diphtheria–tetanus toxoid–pertussis vaccine.

7 developed typical symptoms. Of the 9 immunized children, 8 became infected (confirmed by laboratory tests), but only 1 developed typical symptoms. Six of the 9 immunized children received their first DTP dose at age ≤6 months. Although no difference in the pertussis infection rate was noted between the nonimmunized and immunized groups (7/10 vs. 8/9), the attack rate was 6 of 10 in nonimmunized children and 1 of 6 in immunized children who received their first DTP immunization at age 3–6 months. This finding and the results of the immunogenicity study of DTPa by age suggest strongly that the Japanese DTPa vaccine is equally effective in children ages 3–6 months or 2 years.

The antibody responses to PT after vaccination in the typically symptomatic case were comparable with those in the other 8 immunized children (figure 4A). The PT antibody level in the sera of 9 immunized children obtained just before the pertussis outbreak was lowest in the typically symptomatic case. The interval between the last vaccination and outbreak of pertussis was 25 months in the typically symptomatic case and 6–10 months in the other 8 immunized children. Therefore, the low PT antibody level observed before the pertussis outbreak in the typical case can be explained by the long interval (25 months) between vaccination and disease exposure. The antibody responses to FHA after vaccination and just before the pertussis outbreak were similar to the responses to PT (figure 4B).

Neither leukocytosis nor lymphocytosis were observed in immunized children with pertussis. Immunized children also had less severe disease than did nonimmunized children with pertussis. In this outbreak, the isolation rate of *B. pertussis* in the former was also lower than in the latter (2/9 vs. 6/10). Furthermore, the development of symptoms after exposure to pertussis occurred later in vaccinated children than in symptomatic unvaccinated children (38 vs. ≤28 days in this outbreak), similar to that observed in a Swedish trial [9]. These findings suggest that the effect of the vaccine weakens a few years after vaccination, although disease severity and lymphocytosis are still modified in immunized children.

Several groups have described a decline in whole cell vaccine–induced immunity [10, 11], including my group [12], which reported an outbreak of pertussis in highly immunized adolescents and secondary spread to their families. Immunity induced by DTPw or DTPa vaccines weakens considerably 6–10 years after vaccination. Although the effect of vaccination may be maintained by natural exposure to the disease, the likelihood of this occurring is very low because of the low incidence of pertussis. Therefore, booster immunizations are needed to maintain the protective effect of the vaccine.

### Pertussis in Adults

My colleagues and I surveyed 89 households in which ≥1 person with culture-confirmed pertussis was detected [13]. In 10 households (11.2%), the source of infection was an adult; the secondary attack rate was 19 (10.3%) of 185. Furthermore, a laboratory study disclosed 17 adults with subclinical pertussis (subclinical infection rate, 17 [25%] of 68).

To compare pertussis in adults and children by clinical and hematologic features, 14 adults and 50 children with culture-
confirmed pertussis were selected from the 89 households. Pertussis in adults was generally less severe than in children. Most infected adults reported shortness of breath and a tingling sensation in the throat, whereas many children with pertussis suffered cough-induced vomiting, whoop, and cyanosis, which were rarely observed in adults.

Thirty-two blood samples from 14 adults and 120 samples from 50 children (≤1 year old) with pertussis were examined. Marked leukocytosis and lymphocytosis (leukocyte count ≥15,000/mm³) were observed in the children within 30 days of disease onset, but neither leukocytosis nor lymphocytosis was observed in the adults. Pertussis used to be mainly a childhood disease, but adults are now a major source of its transmission. As the incidence of pertussis among adults has yet to be elucidated, it is difficult to know whether to advocate pertussis immunization for all adults. Adults who work in close contact with children (e.g., in day care centers, schools, and pediatric wards) are more likely to contract pertussis, and immunization of such individuals may be desirable for disease control.

**Summary**

Clinical trials with DTPa and research into pertussis by the Vaccination Research Group of Keio University yielded the following results. After vaccination, the incidence of fever ≥38°C was 2%–4%. Local redness ≥5 cm in diameter was negligible after the first dose and 7%–8% after the second, third, and booster doses. Severe local reactions were seen occasionally (incidence of 1 in every few hundred injections). All local reactions subsided without sequelae. No differences in incidences of fever or other local reactions were observed among different vaccine lots or by vaccine manufacturer. Extrapolation of data from the national compensation system showed an incidence of severe neurologic reactions with sequelae of 0.2/10⁶ doses of DTPa.

The anti-PT antibody responses to the DTPa vaccines were equal to or greater and the anti-FHA antibody responses were far higher in vaccinees than in convalescent patients with pertussis. Although there were significant differences in the antibody responses against pertactin and fimbriae among vaccines from different manufacturers, the DTPa vaccines were equally immunogenic in children ages 3–6 months and 2 years. DTPa vaccines were as effective as DTPw vaccines with an estimated protective efficacy of 84% in children ages 2–8 years (95% CI, 71%–91%). The vaccines appeared to be equally effective in children ≤6 months old and in those ≥2 years old. No differences in the efficacies of the vaccines from different manufacturers were noted. In addition, DTPa did not prevent infection by *B. pertussis* but modified the severity of disease.

Although adult pertussis is usually unrecognized because its clinical and laboratory features differ from those in children, it is a significant health threat that necessitates some disease-control measures.

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**References**


