Antibody Responses to *Bordetella pertussis* Fim2 or Fim3 following Immunization with a Whole-Cell, Two-Component, or Five-Component Acellular Pertussis Vaccine and following Pertussis Disease in Children in Sweden in 1997 and 2007

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*Bordetella pertussis* fimbriae (Fim2 and Fim3) are components of a five-component acellular pertussis vaccine (diphtheria–tetanus–acellular pertussis vaccine [DTaP5]), and antibody responses to fimbriae have been associated with protection. We analyzed the IgG responses to individual Fim2 and Fim3 in sera remaining from a Swedish placebo-controlled efficacy trial that compared a whole-cell vaccine (diphtheria-tetanus-whole-cell pertussis vaccine [DTwP]), a two-component acellular pertussis vaccine (DTaP2), and DTaP5. One month following three doses of the Fim-containing vaccines (DTwP or DTaP5), anti-Fim2 geometric mean IgG concentrations were higher than those for anti-Fim3, with a greater anti-Fim2/anti-Fim3 IgG ratio elicited by DTaP5. We also determined the responses in vaccinated children following an episode of pertussis. Those who received DTaP5 showed a large rise in anti-Fim2 IgG, reflecting the predominant Fim2 serotype at the time. In contrast, those who received DTwP showed an equal rise in anti-Fim2 and anti-Fim3 IgG concentrations, indicating that DTwP may provide a more efficient priming effect for a Fim3 response following contact with *B. pertussis*. Anti-Fim2 and anti-Fim3 IgG concentrations were also determined in samples from two seroprevalence studies conducted in Sweden in 1997, when no pertussis vaccine was used and Fim2 isolates predominated, and in 2007, when either DTaP2 or DTaP3 without fimbriae was used and Fim3 isolates predominated. Very similar distributions of anti-Fim2 and anti-Fim3 IgG concentrations were obtained in 1997 and 2007, except that anti-Fim3 concentrations in 1997 were lower. This observation, together with the numbers of individuals with both anti-Fim2 and anti-Fim3 IgG concentrations, strongly suggests that *B. pertussis* expresses both Fim2 and Fim3 during infection.

*Bordetella pertussis* causes whooping cough, which continues to be a public health concern despite high vaccine coverage. Indeed, the rates of pertussis disease have increased in many countries in recent years, raising the concern that the immunity provided by acellular vaccines may be waning more quickly than that provided by whole-cell pertussis vaccines (1, 2, 3). Thus, it is important to understand the antibody responses induced by the components of acellular pertussis vaccines, particularly those associated with protection.

Fimbriae are important antigens in the pathogenesis of pertussis disease, functioning as adhesins (4). They are built up by subunits to make long filamentous structures on the surface of the bacteria (5). Two different serologically distinct fimbriae, which are composed of either the Fim2 or Fim3 major subunits (22.5 and 22.0 kDa, respectively), are expressed (6). Subtypes of Fim2 (fim2-1 and fim2-2) and Fim3 (fim3-1, fim3-2, and fim3-3) have also been described (7, 8). Fimbriae are present in whole-cell pertussis vaccines, and in 1979, the WHO, supported by epidemiological data, suggested that both Fim2- and Fim3-expressing strains should be included in whole-cell pertussis vaccines (9, 10, 11). Fim2 and Fim3 are also included in a 5-component acellular pertussis vaccine (diphtheria–tetanus–acellular-pertussis vaccine [DTaP5]). The fimbriae are copurified during the vaccine-manufacturing process, and the specific contents of Fim2 and Fim3 have not been defined (12).

In Sweden, the population was not vaccinated against pertussis between 1979 and 1996. During this period, therefore, a number of vaccine trials were performed. In Sweden trial I (13), the efficacies of acellular vaccines composed of two components (DTaP2 containing pertussis toxin [Ptx] and filamentous hemagglutinin [FHA]) or five components (DTaP5 containing Ptx, FHA, pertactin [Prn], and copurified Fim2/Fim3, 5 μg per dose) and a whole-cell vaccine (diphtheria-tetanus-whole-cell pertussis vaccine [DTwP]) were compared. An efficacy of 85.2% was demonstrated for DTaP5, compared to that of a placebo diphtheria-tetanus vaccine (DT) in a 2-, 4-, and 6-month schedule using the WHO-defined criteria that included culture-positive and paired serology-positive cases, with 58.9% and 48.3% efficacies reported for the DTaP2 and DTwP, respectively.

In Sweden trial II (14), a trial without a placebo control, the same two-component vaccine (DTaP2), a three-component vaccine (DTaP3 containing Ptx, FHA, and Prn), a five-component vaccine (DTaP5) and another whole-cell vaccine (DTwP) were compared. It was shown that when mild pertussis disease was included in the case definition, there was significantly lower relative effectiveness of the three-component acellular pertussis vac-
cine not containing fimbriae (DTaP3) compared to that of DTaP5.

An interesting observation arose from the long-term follow-up of children in trial II. The relative effectiveness of DTaP5 has diminished slightly (15) compared to those of both DTwP and the DTaP3 over the same time period. One hypothetical explanation is that the expression of fimbriae in circulating *B. pertussis* changed from predominantly Fim2 during the period when the trials were performed to predominantly Fim3 around 1998-1999 and later. DTaP5 might have been more effective in an environment dominated by Fim2 strains due to stronger immune responses produced by the Fim2 antigen than by the Fim3 antigen in the vaccinated subjects. This hypothesis is supported by the serotype data from about 500 culture-positive children in trial I. There was a statistically significant lower rate of Fim2 isolates among the pertussis cases vaccinated with DTaP5 than in the nonfimbriate group (16), indicating that the Fim2 antigen in DTaP5 was more protective than the Fim3 antigen.

Over the years, there has been much interest concerning the correlation between the antibodies to *B. pertussis* virulence factors and protection. In the early days of pertussis vaccination, agglutinins were used as markers for protection (17). It is now thought that the agglutinins are directed to fimbriae, Prn, and lipopolysaccharide (18). Meade et al. showed that appropriate anti-fimbriate 2/3 enzyme-linked immunosorbent assays (ELISAs) (19) gave results similar to those provided by agglutinin assays in the evaluation of acellular vaccine immunogenicity. The correlations between levels of antibodies to acellular vaccine components and protection against pertussis disease after household exposure to *B. pertussis* were also studied in Sweden vaccine trial I. The correlation between the levels of anti-pertactin antibodies and antibodies against copurified Fim2/3 antigen and disease was clear and statistically significant. A weak protective relationship was revealed for anti-Ptx antibodies (20). Similar results were reported by Cherry et al. in a German household study (18).

Recently, we described the antibody response to Fim2 or Fim3 separately following immunization with DTaP5 (containing 5 μg per dose Fim2/3) or pertussis disease (21). It was found that all individuals showed increases in anti-Fim2 and anti-Fim3 IgG concentrations following vaccination with DTaP5, with 3-fold greater anti-Fim2 than anti-Fim3 IgG concentrations seen in 15-month-old and 4- to 6-year-old children. It was also shown that individuals with evidence of recent pertussis disease (confirmed by Ptx serology) had greater anti-Fim3 than anti-Fim2 IgG concentrations, consistent with the predominant serotype of *B. pertussis* isolates during the sampling period.

In a previous report, we characterized anti-Fim antibodies after immunization at 2, 4, and 6 months of age and after a later episode of pertussis (verified by paired anti-Ptx serology) in 370 sera from 96 participants in Sweden trial I using a copurified anti-Fim2/3 antigen (22). The separate Fim2 and Fim3 antigens have now made it possible to go back and characterize the concentrations of anti-Fim2 and anti-Fim3 IgG separately in the subset of those children with sera still available. In addition, sera from two seroepidemiology collections were also reanalyzed for anti-Fim2 and anti-Fim3 IgG concentrations. The first set of serum samples was collected in 1997 in Sweden just after vaccination against pertussis was reintroduced after a hiatus of 17 years. The children included in this serosurvey were all nonvaccinated, and at this time, Fim2 strains were predominant. The other set of serum samples was collected in 2007 after 10 years’ use of either of the non-Fim vaccines DTaP2 (Pentavac; Sanofi Pasteur MSD) or DTaP3 (Infanrix; SmithKline Beecham) when Fim3 strains predominated (22).

The aims of this study were to determine the specific anti-Fim2 and anti-Fim3 IgG responses after vaccination with either DTwP or DTaP5 and specific anti-Fim IgG profiles before and after disease in children who had received or not received priming vaccinations. An additional aim was to analyze separate anti-Fim2 and anti-Fim3 IgG responses in seroprevalence samples obtained in Sweden in 1997 and 2007, which were positive with the combined anti-Fim2/3 antigen, to determine the effect of the prevailing *B. pertussis* serotype.

**MATERIALS AND METHODS**

**Vaccines and sera used in Sweden trial I (1992-1995).** (i) Vaccines used in Sweden trial I. The vaccines used were (a) the experimental two-component acellular vaccine (DTaP2) (SmithKline Beecham, Rixensart, Belgium [now GlaxoSmithKline]), (b) the experimental five-component acellular vaccine (DTaP5) (Connaught Laboratories, Toronto, Canada [now Sanofi Pasteur]), (c) the whole-cell vaccine (DTwP) licensed in the United States (Connaught Laboratories, Swiftwater, PA), and (d) the placebo vaccine (DT) (SBL-Vaccin AB, Stockholm, Sweden), which contained diptheria and tetanus toxoids. All vaccines were adsorbed to aluminum salts.

(ii) Collection of serum samples in Sweden trial I. Sweden trial I was a placebo-controlled randomized vaccine efficacy trial that enrolled 9,829 children born in 1992 who were immunized at 2, 4, and 6 months of age in one of the four vaccine arms described above (13). Serum samples in this study were obtained from subsets as follows. (a) Preplanned serum samples were collected from almost all of the children in trial I at the predetermined age of 1 year and later at either 2, 2.5, or 3 years of age. (b) Prescheduled serum samples for the immunogenicity studies were also collected systematically at one study site (Linköping, Sweden). Blood samples were routinely collected during Sweden trial I from all children recruited in Linköping at 2 months of age, just before the first trial dose, and thereafter at 7, 13, and 29 months of age, i.e., 1, 7, and 23 months after the third vaccine dose. (c) Acute- and convalescent-phase serum samples, obtained during episodes of suspected pertussis, were analyzed for diagnostic purposes during the pertussis trial I.

(iii) Serum samples in Sweden trial I used in this study. All serum samples obtained 1 month after the third dose of either DTaP5 (182 children) or DTwP (146 children) at 1 of the 14 study sites were analyzed for anti-Fim2 and anti-Fim3 IgG concentrations. In addition, serum samples from infected young children from all four vaccine arms in Sweden trial I were used to study anti-Fim2 and anti-Fim3 IgG concentrations (amplitude, decay, and persistence) before, during, and after a pertussis infection.

For this study, we identified a subset of 91 children who received three trial vaccine doses and had pertussis infections confirmed by anti-Ptx IgG ELISA after the third trial dose according to the criteria in trial I (13) and one or more serum samples obtained before, during, and after the pertussis episode. A total of 334 serum samples still available, three to four per child, were obtained in trial I from the 91 culture-negative but anti-Ptx-positive children and were still available. These samples were reanalyzed for both anti-Fim2 and anti-Fim3 IgG concentrations. Typically, for each child, a 1-year sample was taken before the positive episode, an acute-phase sample and a convalescent-phase sample were taken during the episode, and a 2-, 2.5-, or 3-year blood sample was taken after the end of the pertussis episode. Pre-episode samples for reanalysis existed for 79 children. Serum samples collected after onset of pertussis disease (n = 255) from 91 children were still available. Of these children with pertussis infection during the trial and with blood samples taken after onset of the pertussis episode, 13 were given DTaP5, 16 were given DTwP, 46 were
given DTaP2, and 16 were given DT. Children in the diphtheria-tetanus-pertussis (DTP) groups were regarded as vaccine failures, while the DT group served as a placebo control.

(iv) Vaccines in the universal vaccination program in Sweden after 1996. During the 10-year period after reintroduction of pertussis vaccination, the vaccines used in infancy differed in time and by geographic regions. During 1996 and 1997, a trivalent three-component DTaP3 containing Ptx, FHA, and Prn (Infanrix; SmithKline Beecham) was used in the whole country, except in the Gothenburg area, where a one-component DTaP1, containing Ptx only, was used. From the end of 1998, Infanrix was replaced in a number of counties by a pentavalent two-component DTaP2—inactivated poliovirus vaccine (IPV)—Haemophilus influenzae type b vaccine (Hib) with Ptx and FHA (Pentacel; Sanofi Pasteur MSD).

(v) Seroepidemiology serum samples in 1997 and 2007. In 1997, a seroepidemiology study was conducted in Sweden (22). None of the children in this study had received pertussis vaccine. A similar seroepidemiology study was conducted in 2007 (22). Children sampled in 2007 had not been immunized with pertussis vaccines containing fimbriae. In the age group 2 to 15 years, 184 and 95 serum samples, respectively, which had measurable (≥ 2 ELISA units [EU]/ml) anti-Fim2/3 IgG concentrations, were available for analysis with separate Fim2 and Fim3 antigens. ELISAs for anti-Fim2 and anti-Fim3 IgGs. Indirect ELISA methods were used to measure the concentrations of IgG antibodies to Fim2 and Fim3 separately. General outlines were described in previous publications (23, 24). Fim2 and Fim3 antigens were prepared as described by Alexander et al. (21), and both were treated with 4 M urea before use in the assays. The antigens were diluted to 1.0 mg/liter in phosphate-buffered saline (PBS) (pH 7.4) and were used for to coat plates, which were stored overnight at room temperature. A National Institute for Biological Standards and Control (NIBSC) reference human antiserum (89/530), previously determined to contain 74.1 EU/ml IgG anti-Fim2 and 14.8 EU/ml IgG anti-Fim3, was used as the primary calibrator (21). A pool of fractionated human plasma (IgG) was used as the in-house secondary calibrator. Following parallel assays in duplicate on three different days, IgG2 was assigned values of 2,300 EU/ml for anti-Fim2 and 2,200 EU/ml for anti-Fim3 and diluted 1/100 when used in the assay. The minimum level of detection (MLD) was 2 EU/ml for anti-Fim2 and 1 EU/ml for anti-Fim3. For the analyses of antibody concentrations, 5 EU/ml was used as the cutoff as this value was previously used in a study addressing correlates of protection for anti-Fim2/3 (20).

Statistical methods. Geometric mean antibody concentrations with 95% confidence intervals were calculated. Reverse cumulative distribution curves were plotted as described by Reed et al. (25). The comparisons of groups were performed using the Mann-Whitney test and Minitab version 16.2.2.

RESULTS
Quantification of anti-Fim2 and anti-Fim3 IgG concentrations in sera from Sweden trial I at 7 months of age following vaccination at 2, 4, and 6 months of age. Sera from 182 children who received three doses of DTaP5 and 146 children who received DTwP were analyzed (Table 1). The geometric mean anti-Fim2 IgG concentrations were significantly higher in children who received DTaP5 than in those who received DTwP (P < 0.01). The anti-Fim2 IgG concentrations were greater than those for anti-Fim3 following both vaccines, although the ratio of anti-Fim2/anti-Fim3 was lower in individuals who received DTaP (ratio = 1.6) than in those who received DTwP (ratio = 6.6, P < 0.05). The distribution of anti-Fim2 and anti-Fim3 IgG concentrations obtained is shown in the reverse cumulative distribution curves in Fig. 1. Greater proportions of individuals with higher anti-Fim2 IgG concentrations can be seen for both vaccines, with over half of those who received DTaP having anti-Fim2 IgG concentrations >100 EU/ml. In addition, the gradient of the anti-Fim3 curve for the subjects who received DTwP is steeper than that for the subjects who received DTaP, indicating a more uniform response with DTwP.

Quantification of anti-Fim2 and anti-Fim3 IgG concentrations in sera from Sweden trial I at 12 months of age following vaccination at 2, 4, and 6 months of age. To determine the anti-Fim2 and anti-Fim3 IgG concentrations in vaccinated individuals before the onset of pertussis disease, we identified 79 predisease serum samples, 74 of which were taken at 1 year of age, i.e., at about 6 months after the third vaccine dose. The other 5 were early acute-phase samples collected when the “sample trigger” for a study child, either pertussis disease or suspected pertussis disease in another household member, was present. These samples were taken between 17 and 595 days before the onset of cough and were analyzed for anti-Fim2- and anti-Fim3-specific IgG. The geometric mean concentrations of sera from each vaccine group are shown in Table 2.

There were 13 samples from children vaccinated with DTaP5 and 12 samples from those vaccinated with DTwP. All but one of these samples were taken at 1 year of age. The geometric mean IgG antibody concentrations seen for Fim2 were higher than those for Fim3 following either DTaP5 or DTwP, although this difference was not significant (P > 0.1). However, the anti-Fim2/anti-Fim3 ratio was greater following vaccination with DTaP5 than DTwP (P = 0.03). Only one of the samples in each of the DTaP5 and DTwP groups had a ratio <1.

One child (8%) who received DTaP5 was below the 5 EU/ml cutoff for anti-Fim2 IgG as were two children (17%) who received DTwP. For anti-Fim3 IgG, 69% (n = 9) and 33% (n = 4) of samples were below this cutoff for the DTaP5 and DTwP groups, respectively. Only one sample was below the MLD for both anti-Fim2 and anti-Fim3 IgG following immunization with a Fim-containing vaccine.

Individuals vaccinated with DT or DTaP2, 52 of 54 samples (96%) were previously found to have anti-Fim2/3 IgG concentrations below the protective cutoff of 5 EU/ml (20). Of these, 49 were below the MLD for anti-Fim2, and 29 were below the MLD for anti-Fim3.

Peak anti-Fim2 and anti-Fim3 IgG concentrations determined after vaccination and onset of pertussis disease. A total of 255 samples from 91 children from Sweden trial I following vaccination with either DTaP5, DTaP2, DTwP, or DT were available for quantification of anti-Fim2 and anti-Fim3-specific IgG concentrations after onset of pertussis disease. There were 29 children with 80 samples in the two Fim-containing vaccination groups...
and 62 children with 175 samples in the two Fim-naive groups. The geometric mean concentrations of the peak values obtained for each child are shown in Table 3. The trends are also visualized by reverse cumulative distribution curves of the peak IgG concentrations as shown in Fig. 2, although these are based on only a few cases (n = 13 for DTaP5 and n = 16 for DTwP). It can be seen that the Fim-containing vaccines provide a clear priming effect for both Fim2 and Fim3 with large increases in IgG concentrations for both antigens following disease. In children who received DTaP5, the postdisease peak geometric mean IgG concentration is 11.1-

### TABLE 2

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>No. of children</th>
<th>GM&lt;sup&gt;b&lt;/sup&gt; (EU/ml)</th>
<th>95% confidence interval of GM</th>
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</thead>
<tbody>
<tr>
<td>DTaP5 anti-Fim2</td>
<td>13</td>
<td>14.0</td>
<td>7.1–27.4</td>
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<tr>
<td>DTaP5 anti-Fim3</td>
<td>13</td>
<td>3.8</td>
<td>1.8–8.1</td>
</tr>
<tr>
<td>DTaP5 anti-Fim2/anti-Fim3 ratio</td>
<td>13</td>
<td>3.7</td>
<td>2.0–6.7</td>
</tr>
<tr>
<td>DTwP anti-Fim2</td>
<td>12</td>
<td>12.7</td>
<td>7.1–22.7</td>
</tr>
<tr>
<td>DTwP anti-Fim3</td>
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<td>5.9</td>
<td>3.6–9.8</td>
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<tr>
<td>DTwP anti-Fim2/anti-Fim3 ratio</td>
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<td>2.1</td>
<td>1.3–3.5</td>
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<td>DTaP2 anti-Fim2</td>
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<td>1.1</td>
<td>1.0–1.3</td>
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<tr>
<td>DTaP2 anti-Fim3</td>
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<td>1.0</td>
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<tr>
<td>DT anti-Fim2</td>
<td>12</td>
<td>1.1</td>
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<tr>
<td>DT anti-Fim3</td>
<td>12</td>
<td>0.7</td>
<td>0.5–1.0</td>
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<sup>a</sup>Values below MLD were set to 1 for anti-Fim2 and to 0.5 for anti-Fim3.

<sup>b</sup>GM, geometric mean.

### TABLE 3

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>No. of children</th>
<th>GM&lt;sup&gt;b&lt;/sup&gt; (EU/ml)</th>
<th>95% confidence interval of GM</th>
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<td>111.8–319.7</td>
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<td>11.0</td>
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<td>DTwP anti-Fim2</td>
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<td>118.9</td>
<td>59.8–236.8</td>
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<td>1.4–4.3</td>
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<tr>
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<tr>
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<td>DT anti-Fim2/anti-Fim3 ratio</td>
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<td>0.5</td>
<td>0.4–0.7</td>
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</table>

<sup>a</sup>The subjects listed here represent the 79 subjects in Table 2 plus 12 others for whom the preexposure samples were no longer available.

<sup>b</sup>GM, geometric mean.
fold greater for anti-Fim2 and 4.2-fold greater for anti-Fim3 than the values that were observed at 6 months following vaccination. Those who received DTwP appeared equally primed for anti-Fim2 and anti-Fim3 IgG responses with rises of 9.4- and 8.5-fold, respectively (Table 3, compare to Table 2). Significantly lower anti-Fim2 and anti-Fim3 IgG geometric mean concentrations were seen in children who received the vaccines without fimbriae (DTaP2 and DT, \( P < 0.05 \)) (Table 3) than in the Fim vaccine group.

The reverse cumulative distribution curves of the postvaccination, post-disease samples analyzed for anti-Fim2 and anti-Fim3 IgG concentrations (Fig. 2) clearly show the higher anti-Fim2 IgG concentrations in those who previously received DTaP5 or DTwP than in the Fim-naive group with a steeper gradient of responses evident for DTaP5 recipients, indicating a more uniform response to this antigen with the acellular vaccine. The curves for anti-Fim3 IgG concentrations reinforce the observation that DTwP provides effective priming for the anti-Fim3 response following disease and that the Fim3 component of DTaP5 is inferior in this respect, with similar profiles of postdisease anti-Fim3 IgG concentrations in those who received DT, DTaP2, or DTaP5. Among those not primed with fimbriae, 80% of the samples in the DTaP2 group for anti-Fim2 IgG and 64% for anti-Fim3 IgG were \( > 5 \) EU/ml following the pertussis episode. In the DT group, the corresponding figures were 60% and 56% of samples, respectively. The anti-Fim2 and anti-Fim3 IgG peak responses were also lower for those vaccinated with DTaP2 than for those who received DT, although these differences did not reach significance (DTaP2 versus DT for Fim2, \( P = 0.19 \), and for Fim3, \( P = 0.14 \)).

**Kinetics of the anti-Fim2 and anti-Fim3 IgG responses in young children with disease which occurred after the third DTaP5 or DTwP dose.** For analysis of the kinetics of anti-Fim2 and anti-Fim3 IgG before and after infection, we examined 105...
sera (80 postonset) from 29 children who were positive for pertussis by anti-Ptx IgG ELISAs (3 to 4 sera per child). The first day of cough was defined as the onset of an episode, and the samples were from a period from 595 days before the onset of symptoms until 700 days after the first day of cough. Samples were grouped into 1 of 12 time intervals before or after the onset of cough. The kinetics of the anti-Fim2 and anti-Fim3 IgG concentrations obtained in samples from a period from 595 days before the onset of symptoms until 700 days after the first day of cough. Samples were grouped into 1 of 12 time intervals before or after the onset of cough. The increase in anti-Fim2 IgG commences within the first 2 to 3 weeks of cough, confirming a booster response in the children vaccinated with DTaP5 or DTwP. The maximum values for anti-Fim2 (approximately 150 EU/ml) were reached within 2 months after the onset of cough. The increase in anti-Fim3 antibodies started later, at about 5 weeks after onset, and the median peak value at 2 months after onset of cough was just >25 EU/ml. The increase from preonset to the maximum postonset level was about 10-fold for anti-Fim2 IgG and about 4-fold (and from a lower preonset level) for Fim3 IgG. The decay curve indicates that there was a change from a rapid to a slower decay rate between 3 and 5 months after the first day of cough. Anti-Fim2 IgG concentrations returned to predisease levels at about 18 months after the onset of disease.

**Anti-Fim2 and anti-Fim3 IgG concentrations in seroepidemiology samples obtained in Sweden in 1997 and 2007.** Seroepidemiology samples were obtained in Sweden in 1997, when Fim2 was the predominant *B. pertussis* serotype, from children who had not received pertussis vaccination. Similar samples were also obtained in 2007, when the predominant serotype was Fim3, from children who had received acellular pertussis vaccines that did not contain fimbriae. All children, aged 2 to 15 years, used in this study were selected because they had measurable antibodies against Fim2/3 (22). There is no reason to believe that samples negative for the combined antigen should be positive for the separate Fim2 and Fim3 antigens. There were 184 sera included from 1997 and 95 sera from 2007. Reverse cumulative distribution curves of anti-Fim2 and anti-Fim3 IgG concentrations obtained in samples from these 2 years are shown in Fig. 4. It can be seen that the curves obtained for anti-Fim2 and anti-Fim3 in 2007 and for anti-Fim2 in 1997 are aligned with no significant difference between these values ($P > 0.05$). However, lower anti-Fim3 IgG concentrations were obtained in samples from 1997 ($P < 0.001$).

These data are also presented in Table 1, which shows the numbers and percentages of individuals with anti-Fim2 and anti-Fim3 IgG concentrations in the ranges of 4 to $<14$, 14 to $<100$, or $>100$ EU/ml. This analysis highlights the fact that the same percentages of observations in each range for anti-Fim2 or anti-Fim3 were found in 2007. It also highlights the greater percentage of individuals in 1997 with anti-Fim2 IgG concentrations between 14 and 100 EU/ml (58%) than those with anti-Fim3 (40%, $P < 0.05$).

**DISCUSSION**

*B. pertussis* fimbriae have been shown to be important components of both whole-cell and acellular vaccines, and antibodies to these proteins have been associated with protection in early trials of whole-cell vaccines (by the MRC) and in household exposure studies (18, 20). IgG responses to copurified Fim2/3 have been measured in the clinical trials of acellular pertussis vaccines (13, 26) and many subsequent studies. The predominant serotype expressed by *B. pertussis* isolates has changed in a number of countries during different time periods (27, 28, 29), and the reasons for this are not understood. *B. pertussis* isolates possess fim2 and fim3 genes, and expression is dependent on a run of C residues in the promoter regions of these genes (12). Spontaneous changes in the poly(C) tract length allow or stop expression, but despite this genetic flexibility, the predominant Fim type remains stable over time until a switch in the population occurs. It is not known if this is driven by immune evasion following a rise in anti-Fim2 or anti-Fim3 IgG levels in those infected.

To understand the role of antibodies to Fim2 and Fim3, we purified individual Fim2 and Fim3 from *B. pertussis* and used these to quantify anti-Fim2- and anti-Fim3-specific IgG following vaccination with DTaP5 in individuals with clear evidence of ongoing and recent pertussis disease (21). The ELISA IgG concentration values assigned to standard serum 89/530 in the previous study (21) were determined by aligning the ELISA dose-response curve for a 1:1 mixture of Fim2 and Fim3 with the dose-response curves obtained with this serum and separate Fim2 or Fim3 prep-
arations coated at equal protein concentrations. Thus, the anti-Fim2 and anti-Fim3 IgG concentration values assigned to each serum sample allow comparisons of the IgG antibody responses to these antigens, although the comparisons should be treated with caution as the functional activities of the antibodies are not known. These separate Fim2 and Fim3 preparations have now been used to quantify anti-Fim2 and anti-Fim3 IgG responses in sera from Sweden trial I obtained between 1992 and 1995 (13) and from seroepidemiology samples obtained in Sweden in 1997 and 2007 (22).

A unique serum collection from a well-controlled group of small children, vaccinated with three doses of either DTaP5, DTaP2, DTwP, or placebo DT and assessed previously for anti-Fim2/3 IgG by ELISAs (13), has allowed us to determine anti-Fim2 and anti-Fim3 IgG responses. Anti-Fim2 and anti-Fim3 IgG concentrations were determined in 7-month-old children in the DTaP5 and DTwP groups only. The anti-Fim2 IgG concentrations were greater than the anti-Fim3 concentrations for both vaccines. It is interesting to note that the anti-Fim2 and anti-Fim3 IgG responses following three doses of vaccine were 7- and 14-fold lower than those previously determined in sera from 15-month-old children following a fourth dose of DTaP5 (21). This previous study showed that the ratio of the anti-Fim2 to the anti-Fim3 IgG concentration decreased with additional doses, and this study confirms that this trend continues until after dose three and that the anti-Fim2/anti-Fim3 ratio is greatest in young children. Anti-Fim2 and anti-Fim3 IgG responses following DTwP have not been reported previously and show that this particular whole-cell vaccine elicited lower anti-Fim2 IgG geometric mean titers than DTaP5 ($P < 0.05$) but higher anti-Fim3 IgG geometric mean titers (Table 1) with a lower ratio of anti-Fim2/anti-Fim3 values (1.6) than that of DTaP5 (6.6).

This serum collection also included children with anti-Ptx IgG-confirmed pertussis infection after vaccination with any of the vaccines used, and this allowed both the anti-Fim2 and anti-Fim3 IgG responses to be determined following disease so that the priming effect of these vaccines for anti-Fim2 and anti-Fim3 responses could be determined. The 6-month postvaccination and predisease anti-Fim2 and anti-Fim3 IgG geometric mean concentrations shown in Table 2 were low for the control (DT) and the DTaP2 vaccine without fimbriae. With both DTaP5 and DTwP, anti-Fim2 IgG values were higher than those obtained for anti-Fim3, continuing the trend observed at 7 months of age. These higher anti-Fim2 antibody levels were probably not influenced by

### TABLE 4

<table>
<thead>
<tr>
<th>Data</th>
<th>1997</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Fim2</td>
<td>Anti-Fim3</td>
<td>Anti-Fim2</td>
</tr>
<tr>
<td>Total no. of observations</td>
<td>184</td>
<td>184</td>
</tr>
<tr>
<td>No. (%) of observations with values of 4 to 14 EU/ml</td>
<td>62 (34)</td>
<td>99 (54)</td>
</tr>
<tr>
<td>95% confidence interval of the %</td>
<td>27–41</td>
<td>46–61</td>
</tr>
<tr>
<td>No. (%) of observations with values of 14 to 100 EU/ml</td>
<td>106 (58)</td>
<td>73 (40)</td>
</tr>
<tr>
<td>95% confidence interval of the %</td>
<td>50–65</td>
<td>33–47</td>
</tr>
<tr>
<td>No. (%) of observations with values of &gt;100 EU/ml</td>
<td>10 (5)</td>
<td>7 (4)</td>
</tr>
<tr>
<td>95% confidence interval of the %</td>
<td>3–10</td>
<td>2–8</td>
</tr>
</tbody>
</table>

*The lower-bound value is included in each stratification. This analysis was performed with available serum samples with measurable anti-Fim2/3 IgG. In 1997, 35% of serum samples had measurable anti-Fim2/3 IgG, and 26% had measurable anti-Fim2/3 IgG in 2007. Simultaneous confidence intervals were constructed on the proportions of the samples falling into the categories chosen here, as if they arose from a multinomial distribution (rather than the underlying distribution shown in Fig. 4). This showed significant ($P < 0.01$) differences in the proportions arising from anti-Fim2 and anti-Fim3 IgG concentrations in 1997, complementing the Mann-Whitney test results.

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earlier episodes of subclinical pertussis as the anti-Fim2/3 concentrations seen in the DTaP5 and DTwP subgroups in an earlier study did not differ from those seen in a larger data set of 1-year samples after vaccination (22). The values reported previously for the 15-month-old children in samples obtained before the fourth dose of DTaP5 are comparable to those reported in this study from children 6 months after three doses of DTaP5 (21).

The anti-Fim2 and anti-Fim3 IgG peak levels found in these children in a current episode of pertussis (Table 3 and Fig. 3) show the clear priming effect of prior vaccination with a Fim-containing vaccine. Children who received DTaP5 or DTwP vaccines showed much greater postdisease anti-Fim2 and anti-Fim3 IgG levels than those who previously received DT or DTaP2. The responses to Fim2 were greater than those to Fim3, corresponding with the predominant Fim2 serotype of isolates at this time. However, for interpretation of these data, it must be kept in mind that the distinction between the serotypes of isolates (2, 3 or 2/3) is based on an insensitive agglutination technique. It might be that the technique does not detect small amounts of either Fim3 or Fim2 in vitro. Also, both antigens may be expressed in vivo as suggested by Heikkinen et al. (30).

There is also the possibility that the anti-Fim2 response is more efficiently primed, particularly by DTaP5, possibly due to the greater Fim2 than Fim3 content of this vaccine. It is interesting to observe that the ratio of predisease to peak postdisease anti-Fim2 IgG levels following DTaP5 was greater than that for anti-Fim3 (Table 2 and 3), whereas the pre- to postdisease anti-Fim2 and anti-Fim3 IgG levels were similar in those who had received DTwP. Thus, it appears that DTwP is a more effective priming vaccine for an anti-Fim3 response. This is clearly shown in Fig. 2B, where the reverse cumulative distribution curve for anti-Fim3 IgG levels is clearly shifted to the right for those who had received DTwP. This result is perhaps surprising as most isolates during this time were serotyped as Fim2 but may reflect a proportion of strains expressing Fim3. However, some caution is recommended in drawing conclusions from these data as peak anti-Fim2 and anti-Fim3 IgG responses were determined with sera from only a small number of individuals. The observation that anti-Fim2 IgG responses were greater than those for anti-Fim3 following vaccination with DTaP5 may tie in with a study also performed in Sweden in 1992-1995 that suggested that DTaP5 may be less effective against Fim3 strains as described in the Introduction.

It is clear that the anti-Fim2 and anti-Fim3 responses seen following disease were greatly influenced by previous vaccination, with greater anti-Fim2 and anti-Fim3 responses seen in those who received DTaP5 or DTwP than in those vaccinated with a non-Fim vaccine. It has been shown that previous vaccination can also blunt responses to antigens not included in the vaccine (31). It can be seen in Fig. 2 that the distributions of anti-Fim2 and anti-Fim3 responses are lower in those who received DTaP2 than in those who received the control DT vaccine although this result was not significant with the sample size used. Cherry et al. (31) proposed that this was due to linked epitope suppression caused by preferential responses of memory B-cells following secondary exposure to the vaccine components. The memory B-cells thus outcompete naive B-cells for access to the Bordetella epitopes. An alternative explanation is that some protection is provided by the DTaP2 vaccine and that vaccine failures in the DTaP2 vaccine group may have milder or less prolonged disease than those in the DT group and thus have reduced responses to nonvaccine antigens.

We also had access to seroepidemiology samples collected in 1997 from children who had not received a pertussis vaccination and to samples collected in 2007 when either DTaP2 or DTaP3 had been in use for about 11 years. The reverse cumulative distribution curves in Fig. 4 show that the distributions of the IgG concentrations obtained are the same for anti-Fim2 in both years and for anti-Fim3 in 2007, while lower anti-Fim3 concentrations were obtained in 1997. The close similarity of the IgG concentration distributions was also clear from the percentages of individuals with anti-Fim2 and anti-Fim3 IgG concentrations in various ranges (Table 4). The observation that the distributions were the same shows that individuals had an equal likelihood of exposure to Fim2 or Fim3 in 2007, despite the majority of case isolates serotyped as Fim3 only. This is clear evidence that B. pertussis is able to express both Fim2 and Fim3 during infection. Expression of both fimbrial genes is affected by a poly(C) tract in the promoter region (12) with an optimal poly(C) tract length required for expression. The poly(C) tract length can be altered by slip strand mispairing during DNA replication. This may occur in vivo at a higher rate than previously thought, and this genetic flexibility may be an advantage to the organism.

Thus, in this study, we have characterized the immune responses to Fim2 and Fim3 separately using a panel of sera from Sweden trial I. This has allowed the anti-Fim2 and anti-Fim3 IgG concentrations to be determined following vaccination with either DT, DTwP, DTaP2, or DTaP5. We have also determined the responses in the same children before, during, and following an episode of pertussis disease. Thus, the priming potentials of DTaP5 and DTwP vaccines for anti-Fim2 and anti-Fim3 IgG responses were determined. As expected, due to Fim2 strains predominating at this time, there was a greater response to Fim2 than to Fim3. However, DTwP-vaccinated children produced equal boosts in responses to both Fim2 and Fim3, suggesting that either there were more circulating Fim3 strains than among those isolated and serotyped or this vaccine provides very efficient priming for response to this antigen. This also suggests that there is an opportunity for reformulating acellular vaccines to improve the priming provided by the Fim3 component. The distributions of anti-Fim2 and anti-Fim3 IgG concentrations against both antigens in seroepidemiology samples obtained in 1997 and 2007 were very similar, except that the anti-Fim3 concentrations in 1997 were lower. This observation strongly suggests that B. pertussis expresses Fim2 and Fim3 during infection irrespective of the predominant serotype of the case isolates.

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