CLINICAL AND VACCINE IMMUNOLOGY, May 2010, p. 741–747 Vol. 17, No. 5
1556-6811/10/$12.00 doi:10.1128/CVI.00469-09

Antibody Response Patterns to *Bordetella pertussis* Antigens in Vaccinated (Primed) and Unvaccinated (Unprimed) Young Children with Pertussis▽

James D. Cherry,[1]* Ulrich Heininger,[2] David M. Richards,[3] Jann Storsaeter,[4] Lennart Gustafsson,[5] Margaretha Ljungman,[5] and Hans O. Hallander[5]

David Geffen School of Medicine at UCLA and Mattel Children's Hospital UCLA, Los Angeles, California[1]; Division of Pediatric Infectious Diseases, University Children's Hospital, Basel, Switzerland[2]; Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, California[3]; Norwegian Institute of Public Health, Oslo, Norway[4]; and Swedish Institute for Infectious Disease Control, Stockholm, Sweden[5]

Received 13 November 2009/Returned for modification 4 January 2010/Accepted 16 March 2010

In a previous study, it was found that the antibody response to a nonvaccine pertussis antigen in children who were vaccine failures was reduced compared with the response in nonvaccinated children who had pertussis. In two acellular pertussis vaccine efficacy trials in Sweden, we studied the convalescent-phase enzyme-linked immunosorbent assay (ELISA) geometric mean values (GMVs) in response to pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae (FIM 2/3) in vaccine failures and controls with pertussis. In Germany, the antibody responses to *Bordetella pertussis* antigens PT, FHA, PRN, and FIM-2 were analyzed by ELISA according to time of serum collection after onset of illness in children with pertussis who were vaccine failures or who were previously unvaccinated. Antibody values were also compared by severity of clinical illness. In Sweden, infants who had received a PT toxoid vaccine and who were vaccine failures had a blunted response to the nonvaccine antigen FHA compared with the response in children who had received a PT/FHA vaccine. Similarly, infants who had pertussis and who had received a PT/FHA vaccine had a blunted response to the nonvaccine antigens PRN and FIM 2/3 compared with the response in children who were vaccine failures and who had received a PT, FHA, PRN, and FIM 2/3 vaccine. In Germany, in sera collected from 0 to 15 days after pertussis illness onset, the GMVs for all 4 antigens (PT, FHA, PRN, and FIM-2) were significantly lower in an unvaccinated group than in children who were diphtheria-tetanus-acellular pertussis (DTaP) vaccine failures. In the unvaccinated group, the GMV of the PT antibody rose rapidly over time so that it was similar to that of the DTaP vaccine recipients at the 16- to 30-day period. In contrast, the antibody responses to FHA, PRN, and FIM-2 at all time periods were lower in the diphtheria-tetanus vaccine (DT) recipients than in the DTaP vaccine failures. In both Sweden and Germany, children with less severe illness had lower antibody responses than children with typical pertussis. Our findings indicate that upon exposure and infection, previous vaccinees have more-robust antibody responses to the antigens contained in the vaccine they had received than to *Bordetella* antigens that were not in the vaccine they had received. In addition, over time the antibody responses to FHA, PRN, and FIM-2 were greater in children with vaccine failure (primed subjects) than in unvaccinated children (unprimed subjects) whereas the responses to PT were similar in the primed and unprimed children, as determined from sera collected after 15 days of illness. Our findings lend support to the idea that DTaP vaccines should contain multiple antigens.

In a previous study, it was observed that children who were diphtheria-tetanus-acellular pertussis (DTaP) vaccine failures had a minimal antibody response to the nonvaccine antigen adenylate cyclase toxin (ACT), whereas unvaccinated children had a vigorous response to this antigen (4). Specifically, the convalescent-phase enzyme-linked immunosorbent assay (ELISA) antibody geometric mean value (GMV) in response to ACT in 20 unvaccinated children with pertussis was 872 ELISA units (EU)/ml, whereas the convalescent-phase GMV in 10 DTaP vaccine failures was only 49 EU/ml.

This observation of a blunted antibody response to a nonvaccine antigen in children who were DTaP vaccine failures led us to do a broader retrospective study of patterns of antibody responses to vaccine and nonvaccine antigens in children who were vaccine failures in two vaccine efficacy trials in Sweden (1, 5–9, 22, 24). The initial analysis of data from these two trials led us to do further retrospective analyses of antibody response patterns in diphtheria-tetanus-pertussis (DTP) and DTaP vaccine failures (primed subjects) and in diphtheria-tetanus vaccine (DT) recipients (unprimed subjects). We have examined the convalescent-phase GMVs at various times from illness onset in children in a DTaP vaccine efficacy trial in Germany, and we also have examined convalescent-phase GMVs in the German trial and one of the Swedish trials by severity of pertussis illness in vaccine failures and in unvaccinated children (DT recipients) (5–9, 22, 24).

In addition to the studies presented here, ELISA results for the long-term kinetics of antibodies to pertussis toxin (PT) and fimbriae (FIM 2/3) following infection and vaccination in Swedish children have recently been presented (7, 8).

* Corresponding author. Mailing address: Department of Pediatrics, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave., MDCC 22-442, Los Angeles, CA 90095-1752. Phone: (310) 825-5226. Fax: (310) 206-4764. E-mail: jcherry@mednet.ucla.edu.

▽ Published ahead of print on 24 March 2010.

(The data in this paper were presented in part at the 2006 Pediatric Academic Societies Annual Meeting, San Francisco, CA, 29 April to 2 May 2006; at the Eighth International Symposium, Saga of the Genus *Bordetella*, Paris, France, 7 to 10 November 2006; and at the 108th General Meeting of the American Society for Microbiology, Boston, MA, 1 to 5 June 2008.)

MATERIALS AND METHODS

Studies done in Sweden. Convalescent-phase serum samples were obtained from 107 children with pertussis who were participants in a vaccine efficacy trial done in Sweden in the 1980s (1). There were 25 children who had received an acellular pertussis (aP) vaccine containing PT toxoid and filamentous hemagglutinin (FHA) (JNIH-6), 33 children who had received an aP vaccine containing PT toxoid (JNIH-7), and 49 children who had received a placebo. Antibody responses to PT and FHA were measured by ELISA (23). JNIH-6 contained 3.8 μg protein nitrogen each of the PT antigen and the FHA antigen per dose. JNIH-7 contained 6.0 μg protein nitrogen of PT antigen per dose. Eight-month-old infants received 2 doses of vaccine 2 to 3 months apart. Postvaccination GMVs 2 to 3 months after the second dose were ~77 EU/ml for PT and ~25 EU/ml for FHA for the JNIH-6 group and ~164 EU/ml for PT and <2 EU/ml for FHA for the JNIH-7 group.

In a second pertussis vaccine efficacy trial, done in Sweden in the 1990s, convalescent-phase serum samples were obtained from 137 children in a household-contact substudy who had laboratory-confirmed pertussis (6, 24). There were 53 children who had received DT, 38 children who had received a 2-component (PT and FHA) DTaP vaccine (DTaP-2), 13 children who had received a 5-component (PT, FHA, pertactin [PRN], and FIM 2/3) DTaP vaccine (DTaP-5), and 33 children who had received a diphtheria–tetanus–whole-cell pertussis vaccine (DTwP). DTaP-2 contained 25 μg (each) PT and FHA antigens per dose. DTaP-5 contained 10 μg PT, 5 μg FHA, 5 μg FIM 2/3, and 3 μg PRN per dose. DTwP contained 5.7 protective units per dose. Infants received 3 doses of a vaccine, at 2, 4, and 6 months of age. The ELISA antibody data collected 1 month after the third dose are expressed as reversed distribution curves and not mean values (6). The 50% frequency values for antibodies against the PT antigen were ~62 EU/ml for DTaP-2, ~50 EU/ml for DTaP-5, and <1 EU/ml for DTwP; those for antibodies against the FHA antigen were ~200 EU/ml for DTaP-2, ~40 EU/ml for DTaP-5, and ~10 EU/ml for DTwP; those for antibodies against the PRN antigen were <1 EU/ml for DTaP-2, ~100 EU/ml for DTaP-5, and ~60 EU/ml for DTwP; and those for antibodies against the FIM 2/3 antigen were <1 EU/ml for DTaP-2, ~400 EU/ml for DTaP-5, and ~30 EU/ml for DTwP. The median time interval from cough onset to collection of the convalescent-phase serum sample was 73 days. This time interval did not vary significantly among the four vaccine groups. Antibody titers to PT, FHA, PRN, and FIM 2/3 were measured by ELISA.

In the first Swedish trial, convalescent-phase IgG GMVs against PT and FHA were examined by vaccine group (JNIH-6, JNIH-7, and placebo). In the second trial, convalescent-phase IgG GMVs against PT, FHA, PRN, and FIM 2/3 were examined by vaccine group (DT, DTaP-2, DTaP-5, and DTwP). In addition, in the second Swedish trial the GMVs against each antigen by vaccine group were analyzed by illness severity. The severity of illness is one DTaP-2 vaccinee was not available. "Typical" illness was determined according to the World Health Organization (WHO) case definition (established by an *ad hoc* committee), which required at least 21 consecutive days of paroxysmal cough and a positive culture for *Bordetella pertussis*, a significant increase in IgA or IgG antibody to PT, FHA, or FIM, or a culture-confirmed household contact (26). Less severe cases included all children with laboratory confirmation or a culture-confirmed household contact but with less severe cough illnesses.

ELISA antibody values in the 2 Swedish studies should not be compared because the studies were done during different time periods, using different sources of antigens and different reference sera. Similarly, antibody data between the Swedish and German studies should not be compared. All ELISAs were performed at the Swedish Institute for Infectious Disease Control, Stockholm, Sweden, by using the reference line method (6, 18, 23, 24). All cases were laboratory confirmed and had at least 1 day of cough illness.

The 1980s trial was approved by the ethics committees at the Karolinska Institute and at Umeå, Uppsala, and Linköping Universities (1). Universal subject clearances were obtained from the World Health Organization and the National Institute of Allergy and Infectious Diseases. For the 1990s trial, informed consent was obtained from the parents of all participants (6). The trial was approved by the Ethics Committee at the Karolinska Institute in Stockholm.

TABLE 1. GMVs of IgG antibody to PT and FHA in convalescent-phase sera from previous recipients of JNIH-6, JNIH-7, or placebo who had pertussis due to *B. pertussis* infection

Group (no. of subjects)	GMV, EU/ml (95% CI[a])	
	PT	FHA
JNIH-6 (25)	107.2 (74.7–153.8)	87.1 (60.7–125.0)[b]
JNIH-7 (33)	131.8 (90.6–191.9)	12.9 (8.9–18.6)[c]
Placebo (49)	195.0 (137.2–276.9)	21.9 (14.7–32.5)

[a] 95% CI, 95% confidence interval.
[b] JNIH-6 versus JNIH-7, $P < 0.0001$.
[c] JNIH-7 versus placebo, $P = 0.23$.

Studies done in Germany. From May 1991 to December 1994, a controlled vaccine efficacy trial that included a DTaP vaccine (which contained PT toxoid, FHA, PRN, and FIM-2), DTwP, and a DT control group was carried out (5, 9, 22). Infants in this trial received 4 doses of DTaP vaccine, at 3, 4½, 6, and 15 months of age. The DTaP vaccine contained 3.2 μg PT, 34.4 μg FHA, 1.6 μg PRN, and 0.8 μg FIM-2. Antibody GMVs after the third dose were 13.4 EU/ml for PT, 58.1 EU/ml for FHA, 69.1 EU/ml for PRN, and 8.0 EU/ml for FIM-2. In this trial, there were 238 children with laboratory-confirmed *B. pertussis* illnesses. Of this group, 84 had a case definition consistent with the WHO criteria (26) and 154 had less severe respiratory illness (≥7 days of cough), with the same laboratory and household-contact criteria. For each pertussis group (DT, DTaP, and DTwP), GMVs of IgG antibody to PT, FHA, PRN, and FIM-2 were compared between cases consistent with the WHO definition and those consistent with the definition of less severe illness.

Of the 238 children with pertussis, 231 had "acute-phase" sera available. Of this group, we compared the GMVs for the 4 antigens during four time periods from illness onset between cases in the DT group and the DTaP vaccine group. We could not compare the DT group with the DTwP group because the DTwP group did not contain enough cases. Antibody values were determined by ELISA performed at Wyeth-Lederle Vaccines and Pediatrics by a modification of the parallel line method (2, 3, 9, 10, 13, 15, 22). All cases were laboratory confirmed and had at least 7 days of cough illness.

This study was approved by the ethics committee of the University of Erlangen (Erlangen, Germany) (9, 22).

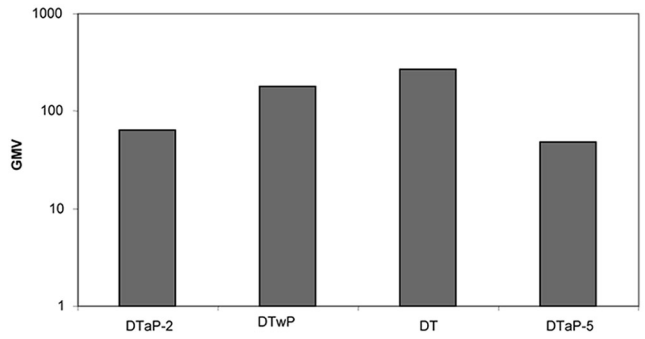
Statistics. Geometric mean values (GMVs) were determined using logarithmic-transformed data, and all values are expressed as GMV in EU/ml or as log GMV. GMVs were estimated and compared using a 2-way analysis of variance (ANOVA) model, with vaccine group and time interval as fixed effects. In one comparison, the paired *t* test was used.

RESULTS

Studies done in Sweden. The GMVs of IgG antibody to PT and FHA in convalescent-phase sera from children with pertussis who were previous recipients of an aP vaccine containing PT and FHA (JNIH-6), an aP vaccine containing PT (JNIH-7), or a placebo in the first Swedish trial are presented in Table 1. The vaccine failures (primed subjects) who had received the PT/FHA vaccine (JNIH-6) had a good antibody response to both antigens, whereas the vaccine failures who had received the PT toxoid vaccine (JNIH-7) had a similar good response to PT but very little response to the nonvaccine antigen FHA. The placebo recipients (unprimed) had a strong antibody response to PT but a minimal response to FHA. The antibody response to FHA in the placebo recipients (21.9 EU/ml) appeared greater than the antibody response in the vaccine failures who had received the PT toxoid vaccine (12.9 EU/ml), but this difference did not reach statistical significance ($P = 0.23$).

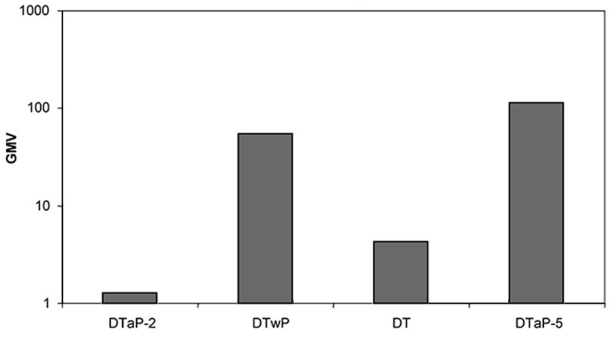
The GMVs of IgG antibody to PT, FHA, PRN, and FIM 2/3 in

A



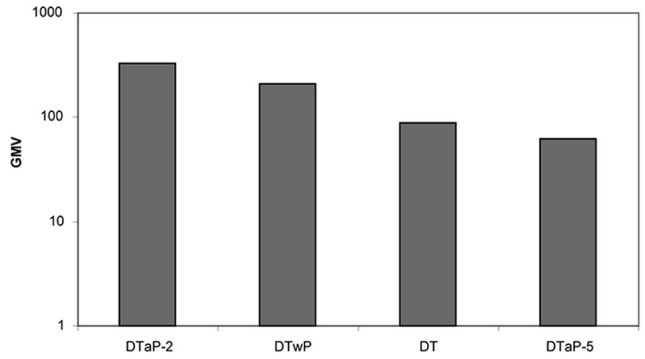
Vaccine group	p values vs vaccine group	p value
DTaP-2	DTwP	<.0001
DTaP-2	DT	0.0002
DTaP-2	DTaP-5	<.0001
DTwP	DT	<.0001
DTwP	DTaP-5	0.0879
DT	DTaP-5	<.0001

C



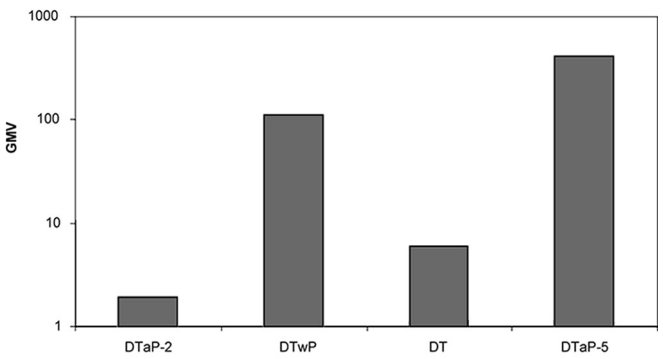
vaccine group	p values vs vaccine group	p value
DTaP-2	DTwP	<.0001
DTaP-2	DT	0.0002
DTaP-2	DTaP-5	<.0001
DTwP	DT	<.0001
DTwP	DTaP-5	0.0879
DT	DTaP-5	<.0001

B



Vaccine group	p values vs vaccine group	p value
DTaP-2	DTwP	0.1125
DTaP-2	DT	<.0001
DTaP-2	DTaP-5	<.0001
DTwP	DT	0.0023
DTwP	DTaP-5	0.0004
DT	DTaP-5	0.2589

D



vaccine group	p values vs vaccine group	p value
DTaP-2	DTwP	<.0001
DTaP-2	DT	0.0016
DTaP-2	DTaP-5	<.0001
DTwP	DT	<.0001
DTwP	DTaP-5	0.0002
DT	DTaP-5	<.0001

FIG. 1. Convalescent-phase GMVs of IgG antibody to PT, FHA, PRN, and FIM 2/3 in 13 DTaP-5, 38 DTaP-2, and 33 DTwP vaccine failures and in 53 controls (DT recipients) in the second Swedish study.

convalescent-phase sera from children with pertussis who were previous recipients of DTaP-2, DTaP-5, DTwP, or DT in the second Swedish trial are presented in Fig. 1. As noted, the DTaP-5 and DTwP vaccine failures had vigorous ELISA antibody responses to all four vaccine antigens (PT, FHA, PRN, and FIM 2/3). In contrast, the DTaP-2 vaccine failures had similar vigorous responses to only the two vaccine antigens (PT and FHA) for which they were primed. The controls (DT recipients) had mod-

TABLE 2. IgG GMVs against PT, FHA, PRN, and FIM 2/3 in Swedish study participants, assorted by vaccine group and analyzed by illness severity

Vaccine group	Antigen	GMV, EU/ml		*P* value
		Less severe illness[a]	Typical illness[a]	
DT	PT	311.3	372.8	0.53
	FHA	58.9	115.0	0.18
	PRN	2.6	6.2	0.16
	FIM 2/3	6.2	7.8	0.71
DTaP-2	PT	74.7	127.6	0.06
	FHA	298.1	432.3	0.28
	PRN	0.6	1.7	0.02
	FIM 2/3	2.5	2.4	0.95
DTaP-5	PT	76.8	204.6	0.06
	FHA	84.1	204.2	0.22
	PRN	135.7	435.4	0.05
	FIM 2/3	304.9	709.3	0.15
DTwP	PT	313.0	304.7	0.94
	FHA	294.8	295.6	0.10
	PRN	103.8	66.7	0.56
	FIM 2/3	228.3	86.4	0.24

[a] Typical, criteria established by an *ad hoc* committee from WHO, which required at least 21 consecutive days of paroxysmal cough and a positive culture for *B. pertussis*, a significant increase in IgA or IgG antibody to PT, FHA, or FIM, or a culture-confirmed household contact (26); less severe, laboratory confirmation or culture-confirmed household contact but with cough illness that did not include 21 consecutive days of paroxysmal cough. For the DT group, 12 subjects had less severe symptoms and 41 had typical symptoms; for the DTaP-2 group, 15 subjects had less severe symptoms and 22 had typical symptoms; for the DTaP-5 group, 6 subjects had less severe symptoms and 7 had typical symptoms; and for the DTwP group, 10 subjects had less severe symptoms and 23 had typical symptoms.

est responses to PRN and FIM 2/3, but nevertheless their convalescent-phase GMVs of antibody to these two antigens were significantly greater then the GMVs in the DTaP-2 vaccine failures ($P = 0.0002$ and $P = 0.0016$, respectively).

It is of interest to note that the antibody response to PT in the unprimed DT vaccinees was significantly greater than that in the DTaP-2 or DTaP-5 vaccine failures ($P < 0.0001$).

Also of note is the antibody response to FHA in the unprimed group (DT recipients) compared to the responses in the three primed groups (DTaP-2, DTwP, and DTaP-5 recipients). The vaccine failures who were primed with DTaP-2 or DTwP had significantly greater GMVs of antibody to FHA than the unprimed group (DT recipients); however, the FHA antibody responses were similar in the DTaP-5 vaccine failures and the placebo recipients (DT vaccinees).

Presented in Table 2 are IgG GMVs against the 4 *B. pertussis* antigens, assorted by vaccine group and analyzed by illness severity. For DT, DTaP-2, and DTaP-5, the GMVs in the typical cases tend to be higher than the GMVs in the children with less severe illness. However, in only 2 of the 12 comparisons was a *P* value of ≥0.5 reached. In the DTwP group, the GMVs appear to be lower in the infants with typical pertussis than in those with less severe illness ($P > 0.05$ in all comparisons).

Studies done in Germany. Presented in Fig. 2 are the GMVs of antibody to PT, FHA, PRN, and FIM-2 during 4 time periods from illness onset in DTaP vaccine failures and in nonvaccinated children (DT recipients).

For sera collected from 0 to 15 days after illness onset, the GMVs for all four antigens were significantly lower in the unvaccinated group than in children who had received the DTaP

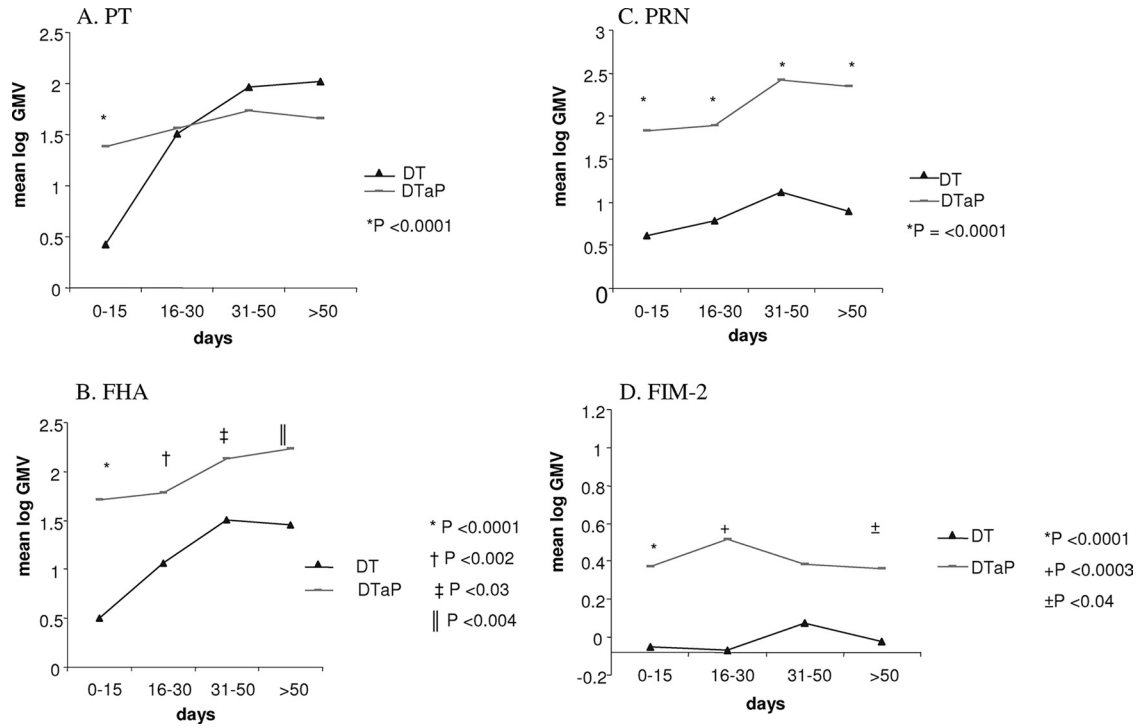


FIG. 2. GMVs of antibody to PT, FHA, PRN, and FIM-2 during selected time periods after illness onset in DTaP vaccine failures and controls (DT recipients) in the German trial.

TABLE 3. IgG GMVs against PT, FHA, PRN, and FIM-2 in German trial participants, assorted by vaccine group and analyzed by illness severity

Vaccine group	Antigen	GMV, EU/ml		*P* value
		Less severe illness[a]	Typical illness[a]	
DT	PT	36.9	198.4	0.002
	FHA	16.5	59.9	0.022
	PRN	7.1	12.5	0.185
	FIM-2	1.2	1.1	0.735
DTaP	PT	49.9	157.8	0.002
	FHA	126.6	199.5	0.162
	PRN	186.2	358.2	0.065
	FIM-2	3.2	4.8	0.231
DTwP	PT	62.2	100.1	0.284
	FHA	17.6	57.3	0.029
	PRN	51.8	140.1	0.037
	FIM-2	7.9	19.5	0.080

[a] Typical, see footnote to Table 2 for description of WHO criteria; less severe, ≥7 days of cough, with the same laboratory and household-contact criteria given in the WHO definition. For the DT group, 12 subjects had less severe symptoms and 89 had typical symptoms; for the DTaP vaccine group, 39 subjects had less severe symptoms and 44 had typical symptoms; and for the DTwP group, 30 subjects had less severe symptoms and 18 had typical symptoms.

vaccine. In the unvaccinated group, the GMV of the PT antibody rose rapidly over time so that it was similar to that for the DTaP vaccine recipients at the 16- to 30-day period (32.1 versus 36.8 EU/ml, respectively [$P = 0.8$]), and at the 31- to 50-day and >50-day periods it appears higher than the GMV of the DTaP vaccine group (92.2 versus 53.9 EU/ml [$P = 0.5$] and 106.3 versus 45.8 EU/ml [$P = 0.2$], respectively). The FHA antibody pattern rise was less marked than that of PT in the unvaccinated subjects, and its peak was lower than that for the DTaP vaccine recipients (28.3 versus 171.7 EU/ml, respectively [$P = 0.004$]). The PRN antibody pattern in the unvaccinated group was less marked than that in the DTaP vaccine group, and the peak titer was >10-fold lower (13.2 versus 266.0 EU/ml, respectively [$P < 0.0001$]). The response to FIM-2 was delayed and minimal in the unvaccinated children compared with that for the DTaP vaccinees.

Presented in Table 3 are the IgG GMVs against the 4 *B. pertussis* antigens, assorted by vaccine group and analyzed by illness severity. In all vaccine categories, the children with more severe illness tended to have higher GMVs than the children with less severe illness. In 5 of the 12 comparisons, the *P* values were <0.04.

DISCUSSION

In a previous study, it was observed that children who were DTaP vaccine failures had a blunted antibody response to the nonvaccine antigen ACT, whereas unvaccinated children with pertussis had a vigorous antibody response to this antigen (4). This observation led us to perform two retrospective studies of vaccine failure or primary infection in two Swedish vaccine efficacy trials (1, 6, 24). Specifically, we have looked at and presented here the antibody responses to vaccine and nonvaccine antigens (FHA, PRN, and FIM 2/3) in vaccine failures and in unvaccinated children. The results of these two analyses led us to perform an additional retrospective study in Sweden and two retrospective studies of German children who participated in a DTaP vaccine efficacy trial (5, 6, 9, 22).

The results of the two retrospective analyses in Sweden indicated that children who were vaccine failures had brisk antibody responses to antigens present in the vaccines they had previously received, whereas their antibody responses to nonvaccine antigens were minimal. In the first Swedish trial, there was a suggestion that the antibody response to the nonvaccine antigen FHA in vaccine failures was lower than the response to the same antigen in nonvaccinated controls with pertussis. In the second Swedish trial, the antibody responses to the two nonvaccine antigens PRN and FIM 2/3 in vaccine failures were significantly lower than the responses to these antigens in the children with pertussis who were DT recipients (controls).

The blunted antibody response to nonvaccine antigens in the children with vaccine failure might be explained simply by the effect of priming versus nonpriming (11). However, this does not explain the fact that the response to the nonvaccine antigens in the vaccine failures was lower than that which occurred in previously unvaccinated children with pertussis. There are perhaps two mechanisms which could explain the minimal response to the nonvaccine antigens in the children who were vaccine failures.

One explanation might be that illness in vaccine failures tends to be less severe, which might result in a lowered antibody response. To shed light on this hypothesis, we carried out the two analyses presented in Tables 2 and 3. Although the findings in both data sets suggest that more severe illness is associated with a more vigorous antibody response, the differences overall are not striking.

A second hypothesis, which we favor, is that the findings can be explained by linked epitope suppression caused by preferential responses of memory B cells following secondary exposure to vaccine components (16). Memory B cells and circulating antibodies are readily available to respond to additional exposures. They outcompete naïve B cells for access to the *Bordetella* epitopes, as they are more numerous and their receptors exhibit a higher antigen affinity. Higher expression levels of cell surface major histocompatibility complex class II (MHC-II) and costimulatory molecules also allow memory B cells to preferentially interact with T helper cells required for further propagation of the immune response. Linked epitope suppression applies as the immune response to the new epitopes is suppressed by the strong response to the original vaccine components.

We believe that the blunted antibody response that we have demonstrated is an important consideration in the choice of DTaP vaccines for general use and for the development of new DTaP vaccines. For example, as data from our two previous studies (5, 24) of serologic correlates of immunity indicate, children who received only a PT toxoid or PT/FHA vaccine have enhanced and continued susceptibility to *B. pertussis* infections compared to that of children immunized with multicomponent vaccines. As modest PRN antibody values are most important for exposed children, failure to include PRN in vaccines likely results in subsequent suppression of PRN responses. The original exposure essentially "locks in" the immune response to certain epitopes and inhibits the response to linked epitopes even following subsequent exposures. The only

way to break this pattern is to expose the individual to new epitopes that are unlinked to the epitopes to which the individual had been exposed in prior encounters. Thus, if immunization against additional epitopes is desired, these epitopes have to be introduced separately in a vaccine to take effect, because in combination vaccines, the generation of antibodies against them is always suppressed due to the preferential response to previously introduced antigens.

Because the antibody responses to PRN and FIM 2/3 in primary infections in controls (DT recipients) were modest in the second study in Sweden, we performed the time-related-response study of the children in the German efficacy trial; we thought that the inferior responses in the controls might be due to the time of collection of the convalescent-phase serum samples. However, this does not appear to be the case (Fig. 2). Of interest are the differences in the patterns of antibody response to PT in the vaccine failures and controls compared with the patterns of response to the other antigens. In the >50-day time period, the GMV response to PT in the control group was similar to or perhaps higher than that in the vaccine failures (106.3 versus 45.8 EU/ml, respectively [$P = 0.2$]). In a study in Senegal, Simondon et al. (21) noted similar findings with children who were vaccine failures or who were unvaccinated. In contrast, in our study of the >50-day period, the responses to FHA, PRN, and FIM-2 were significantly lower in the controls than in the vaccine failures.

The DTaP vaccine failure group has a memory response against specific *Bordetella* antigens, while the DT group is considered naïve. Memory responses are characterized primarily as faster, not necessarily higher, at all time points (11). Therefore, we expect the antibody responses to these *Bordetella* antigens to initially be higher in the DTaP vaccine group than in the DT group but for the DT group's antibody values to eventually catch up. This result occurred with antibody to PT but not with antibody to FHA, PRN, or FIM-2. This difference in the response to PT compared with the responses to FHA, PRN, and FIM-2 might be explained by the uniqueness of PT. Specifically, PT is unique in nature, with no homologues, whereas the other antigens have many homologues in other bacterial species which infect children (14, 25).

Therefore, the DT group could have prior exposure to other bacteria that express FHA-, PRN-, and FIM-like proteins that could bias the immune response away from the *B. pertussis* detectable form of each antigen. This again could be a manifestation of linked epitope suppression. Specifically, the children would have a major antibody response to the proteins like FHA, PRN, and FIM from other bacteria and a lower response to the *B. pertussis*-specific proteins that our ELISAs detect. Since PT is soluble, it could be "seen" by the immune system as a nonlinked antigen (14).

Conclusions. Upon subsequent exposure and infection, previous DTwP or DTaP vaccinees respond more vigorously to the antigens contained in the vaccines with which they were immunized than to other *B. pertussis* proteins, as antibodies to more than one vaccine antigen correlate with protection. Our present findings as well as our previous serologic-correlate data suggest that DTaP vaccines should contain multiple antigens, rather than just PT or PT and FHA (5, 24). Since in five trials DTwP vaccines were more efficacious than DTaP vaccines (9, 12, 17, 19, 20, 22), we should reexamine the antibody responses of DTwP recipients to other antigens (such as lipopolysaccharide [LPS], BrkA, SphB1, Vag8, Bsp22, and TcfA) and explore whether one or more of these antigens as well as ACT should be added to DTaP vaccines.

ACKNOWLEDGMENTS

For studies done in Sweden, the data were obtained from a 1980s study and a 1990s study. The 1980s trial was funded by the Swedish National Bacteriological Laboratory, the Centers for Disease Control (contract 200-85-082), and the National Institute of Allergy and Infectious Diseases (contract N01-A1-62527). The 1990s study was supported by a contract (N01-A1-15125) with the National Institute of Allergy and Infectious Diseases. Statistical analyses for the publication were funded by an unrestricted grant from Sanofi Pasteur.

J.S. was previously employed by GlaxoSmithKline. H.O.H. was a consultant to GlaxoSmithKline.

For studies done in Germany, the trial was funded by Wyeth-Lederle Vaccines and Pediatrics. Statistical analyses for this publication were funded by an unrestricted grant from Sanofi Pasteur.

J.D.C. is a member of the Speakers Bureau of Sanofi Pasteur and GlaxoSmithKline and also has been a consultant to Sanofi Pasteur and GlaxoSmithKline. U.H. has given talks funded by Sanofi Pasteur and GlaxoSmithKline.

We thank Jeffrey Gornbein (Department of Biomathematics, David Geffen School of Medicine at UCLA) and his associates (Rebecca Radbod and Daniela Markovic) for statistical help.

REFERENCES

1. **Ad Hoc Group for the Study of Pertussis Vaccines.** 1988. Placebo-controlled trial of two acellular pertussis vaccines in Sweden—protective efficacy and adverse events. Lancet **i:**955–960.
2. **Blumberg, D. A., C. M. Mink, J. D. Cherry, K. S. Reisinger, M. M. Blatter, B. L. Congeni, C. L. Dekker, M. G. Stout, J. R. Mezzatesta, J. V. Scott, et al.** 1990. Comparison of an acellular pertussis-component diphtheria-tetanus-pertussis (DTP) vaccine with a whole-cell pertussis-component DTP vaccine in 17- to 24-month-old children, with measurement of 69-kilodalton outer membrane protein antibody. J. Pediatr. **117:**46–51.
3. **Blumberg, D. A., C. M. Mink, J. D. Cherry, C. Johnson, R. Garber, S. A. Plotkin, B. Watson, G. A. Ballanco, R. S. Daum, B. Sullivan, et al.** 1991. Comparison of acellular and whole-cell pertussis-component diphtheria-tetanus-pertussis vaccines in infants. The APDT Vaccine Study Group. J. Pediatr. **119:**194–204.
4. **Cherry, J. D., D. X. L. Xing, P. Newland, K. Patel, U. Heininger, and M. J. Corbel.** 2004. Determination of serum antibody to *Bordetella pertussis* adenylate cyclase toxin in vaccinated and unvaccinated children and in children and adults with pertussis. Clin. Infect. Dis. **38:**502–507.
5. **Cherry, J. D., J. Gornbein, U. Heininger, and K. Stehr.** 1998. A search for serologic correlates of immunity to *Bordetella pertussis* cough illnesses. Vaccine **16:**1901–1906.
6. **Gustafsson, L., H. O. Hallander, P. Olin, E. Reizenstein, and J. Storsaeter.** 1996. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. N. Engl. J. Med. **334:**349–355.
7. **Hallander, H. O., M. Ljungman, J. Storsaeter, and L. Gustafsson.** 2009. Kinetics and sensitivity of ELISA IgG pertussis antitoxin after infection and vaccination with *Bordetella pertussis* in young children. APMIS **117:**797–807.
8. **Hallander, H. O., M. Ljungman, M. Jahnmatz, J. Storsaeter, L. Nilsson, and L. Gustafsson.** 2009. Should fimbriae be included in pertussis vaccines? Studies on ELISA IgG anti-Fim 2/3 antibodies after vaccination and infection. APMIS **117:**660–671.
9. **Heininger, U., J. D. Cherry, K. Stehr, S. Schmitt-Grohé, M. Uberall, S. Laussucq, T. Eckhardt, M. Meyer, J. Gornbein, and the Pertussis Vaccine Study Group.** 1998. Comparative efficacy of the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine and Lederle whole-cell component DTP vaccine in German children after household exposure. Pediatrics **102:**546–553.
10. **Heininger, U., J. D. Cherry, P. D. Christenson, T. Eckhardt, U. Goering, P. Jakob, W. Kasper, D. Schweingel, S. Laussucq, J. G. Hackell, et al.** 1994. Comparative study of Lederle/Takeda acellular and Lederle whole-cell pertussis-component diphtheria-tetanus-pertussis vaccines in infants in Germany. Vaccine **12:**81–86.
11. **Janeway, C. A. J., P. Travers, M. Walport, and J. D. Capra.** 1999. Immunological memory, p. 402–413. *In* P. Austin and E. Lawrence (ed.), Immunobiology: the immune system in health and disease, 4th ed. Elsevier, New York, NY.
12. **Liese, J. G., C. K. Meschievitz, E. Harzer, J. Froeschle, P. Hosbach, J. E. Hoppe, F. Porter, S. Stojanov, K. Niinivaara, A. M. Walker, and B. H.**

Belohradsky. 1997. Efficacy of a two-component acellular pertussis vaccine in infants. Pediatr. Infect. Dis. J. **16:**1038–1044.
13. **Manclark, C. R., B. D. Meade, and D. G. Burstyn.** 1986. Serologic response to *Bordetella pertussis*, p. 388–394. *In* N. R. Rose, H. Friedman, and J. L. Fahey (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, DC.
14. **Mattoo, S., and J. D. Cherry.** 2005. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella* subspecies. Clin. Microbiol. Rev. **18:**326–382.
15. **Meade, B. D., C. M. Mink, and C. R. Manclark.** 1990. Serodiagnosis of pertussis, p. 322–329. *In* C. R. Manclark (ed.), Proceedings of the Sixth International Symposium on Pertussis. DHHS publication no. (FDA) 90-1164. Department of Health and Human Services, United States Public Health Service, Bethesda, MD.
16. **Murphy, K. M., P. Travers, and M. Walport.** 2008. Janeway's immunobiology, 7th ed. Garland Science, New York, NY.
17. **Olin, P., F. Rasmussen, L. Gustafsson, H. O. Hallander, and H. Heijbel.** 1997. Randomised controlled trial of two-component, three-component, and five-component acellular pertussis vaccines compared with whole-cell pertussis vaccine. Ad Hoc Group for the Study of Pertussis Vaccines. Lancet **350:**1569–1577.
18. **Reizenstein, E., H. O. Hallander, W. C. Blackwelder, I. Kuhn, M. Ljungman, and R. Möllby.** 1995. Comparison of five calculation modes for antibody ELISA procedures using pertussis serology as a model. J. Immunol. Methods **183:**279–290.
19. **Schmitt, H. J., C. H. Wirsing von König, A. Neiss, H. Bogaerts, H. Bock, H. Schulte-Wissermann, M. Gahr, R. Schult, J. U. Folkens, W. Rauh, and R. Clemens.** 1996. Efficacy of acellular pertussis vaccine in early childhood after household exposure. JAMA **275:**37–41.
20. **Simondon, F., M. P. Preziosi, A. Yam, C. T. Kane, L. Chabirand, I. Iteman, G. Sanden, S. Mboup, A. Hoffenbach, K. Knudsen, N. Guiso, S. Wassilak, and M. Cadoz.** 1997. A randomized double-blind trial comparing a two-component acellular to a whole-cell pertussis vaccine in Senegal. Vaccine **15:**1606–1612.
21. **Simondon, F., I. Iteman, M. P. Preziosi, A. Yam, and N. Guiso.** 1998. Evaluation of an immunoglobulin G enzyme-linked immunosorbent assay for pertussis toxin and filamentous hemagglutinin in diagnosis of pertussis in Senegal. Clin. Diagn. Lab. Immunol. **5:**130–134.
22. **Stehr, K., J. D. Cherry, U. Heininger, S. Schmitt-Grohe, M. Uberall, S. Laussucq, T. Eckhardt, M. Meyer, R. Engelhardt, and P. Christenson.** 1998. A comparative efficacy trial in Germany in infants who received either the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine, the Lederle whole-cell component DTP vaccine, or DT vaccine. Pediatrics **101:** 1–11.
23. **Storsaeter, J., H. O. Hallander, and C. P. Farrington.** 1990. Evaluation of laboratory methods used for the diagnosis of pertussis infection and disease in the Swedish efficacy trial, p. 291–294. *In* C. R. Manclark (ed.), Proceedings of the Sixth International Symposium on Pertussis. DHHS publication no. (FDA) 90-1164. Department of Health and Human Services, United States Public Health Service, Bethesda, MD.
24. **Storsaeter, J., H. O. Hallander, L. Gustafsson, and P. Olin.** 1998. Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. Vaccine **16:**1907–1916.
25. **Vincent, J. M., J. D. Cherry, W. F. Nauschuetz, et al.** 2000. Prolonged afebrile non-productive cough illnesses in American soldiers in Korea: a serologic search for causation. Clin. Infect. Dis. **30:**534–539.
26. **World Health Organization.** 1991. WHO meeting on case definition of pertussis, Geneva, 10–11 January 1991, p. 4–5. MIM/EPI/PERT/91.1. WHO, Geneva, Switzerland. http://whqlibdoc.who.int/hq/1991/MIM_EPI_PERT_91.1.pdf.