MAJOR ARTICLE

Immune Responses and Antibody Decay after Immunization of Adolescents and Adults with an Acellular Pertussis Vaccine: The APERT Study

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As part of a prospective acellular pertussis (ACP) vaccine efficacy trial, 5 serum samples were obtained, over an 18-month period, from 101 ACP-vaccine recipients and 99 control subjects, to assess ACP antibody response and decay. Immunoglobulin (Ig) G and IgA antibodies to pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3 (FIM) were measured by enzyme-linked immunosorbant assay, and titers of agglutinin were determined. Of the subjects, 16%–19% had preimmunization values of antibodies to PT that were above the assay's limit of quantitation (LOQ); in contrast, 36%–63% of the subjects had preimmunization values of antibodies to FHA, PRN, or FIM that were above the LOQ. Substantial increases in titers of IgG and IgA antibodies to the 3 ACP antigens (PT, FHA, and PRN) were observed. Over the 18-months, the percent decay in IgG and IgA antibodies ranged from 56% to 73% and from 57% to 70%, respectively; the IgG antibody response and decay suggests that geometric mean titers likely remain above the LOQ for 2–9 years and above the threshold of detection for 4–13 years. These findings support the use of ACP booster immunizations for adolescents and adults, to provide sustained levels of antibody.

Pertussis vaccines have been in routine pediatric use for 50 years and have dramatically decreased the incidence of whooping cough [1]. Nonetheless, pertussis still occurs in unimmunized infants [1–3]. They experience the greatest morbidity and mortality and often acquire their infections from exposure to older infected persons in the household [1–10]. Neither vaccine nor natural infection induces long-lived immunity [1, 6, 9, 11–13]; consequently, reinfections occur in adolescents and adults and can, in clinical presentation, be asymptomatic, mildly symptomatic, or classic [1, 6–9, 11, 12, 14–18]. In the United States, an increasing proportion of reported pertussis cases occurs in adolescents and adults [1–3]. A prospective, US population–based study with active surveillance has estimated that the annual incidence of pertussis in persons >15 years of age is 507 cases/1,000,000 person-years [16], which extrapolates to >1,000,000 cases or episodes in older persons annually.

Diphtheria-tetanus toxoids–acellular pertussis (DTaP) vaccines are now used routinely in children in North America and in many countries worldwide. The decreased reactogenicity of DTaP vaccines, compared with killed whole-cell pertussis vaccines, permits the consideration of the use of acellular pertussis (ACP) vac-

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cines in adults; such use may bring about major reductions in morbidity and health-care costs for older persons as well as reduce the reservoir of infection and, thus, transmission to young children [1, 9, 11, 19, 20].

The present study evaluates serum samples obtained from the participants in a prospective, multicenter, randomized, double-blind, controlled trial that was sponsored by the National Institutes of Health and that evaluated the reactogenicity, immunogenicity, and protective efficacy of a 3-component ACP vaccine in subjects 15–65 years of age. Multiple blood samples were obtained from 200 subjects over an 18-month period, permitting a detailed characterization of both preexisting and vaccine-induced immunity as well as of antibody decay.

SUBJECTS, MATERIALS, AND METHODS

Subject population. Between July 1997 and December 1998, a double-blind, randomized trial was conducted at 8 US study sites, to evaluate the reactogenicity, immunogenicity, and protective efficacy of an ACP vaccine. A total of 2781 healthy adolescents and adults 15–65 years of age were prospectively enrolled and were randomized to receive an intramuscular dose of either ACP vaccine (GlaxoSmithKlein [GSK]) or control vaccine (Havrix, a GSK hepatitis A vaccine). The first 25 subjects at each of the 8 study sites (a total of 200 subjects) provided 5 serum samples each over an 18-month period: (1) on the day of immunization, before vaccination; (2) at 1 month after immunization; (3) at 6 months after immunization; (4) at 12 months after immunization; and (5) at 18 months after immunization. Study sites included Baylor College of Medicine (Houston, TX), Cincinnati Children's Hospital (Cincinnati, OH), Saint Louis University (St. Louis, MO), the University of Maryland (Baltimore), the University of Rochester (Rochester, NY), Vanderbilt University (Nashville, TN), the University of Pittsburgh (Pittsburgh, PA), and the UCLA Center for Vaccine Research (Torrance, CA).

Of the 200 subjects in our substudy, 101 received the ACP vaccine and 99 received the control vaccine. As a group, they were similar to the entire population of APERT subjects with regard to age (mean, 37.8 years [range, 15–65 years]), sex (33% were male), ethnicity (83% were white, 12% were African American, and 5% were other), study site (25 subjects/site), and occupation (21% were students, 32% were health-care workers, and 47% were community volunteers). Data on antibodies could be obtained at all 5 time points for 93% of the subjects in the present study.

ACP and control (hepatitis A) vaccines. The ACP-vaccine formulation administered in our study contained 8 μg each of pertussis toxin (PT) and filamentous hemagglutinin (FHA) and 2.5 μg of pertactin (PRN), one-third of the content of the licensed GSK pediatric 3-component DTaP vaccine (Infanrix and Pediarix, GSK) [21]. It had no diphtheria or tetanus components.

Havrix, a licensed GSK inactivated hepatitis A vaccine, was the blinded control vaccine. It was administered as a single, 0.5-mL dose (720 ELISA units) and was visually indistinguishable from the ACP vaccine.

Serologic studies. IgA and IgG antibodies to PT, FHA, PRN, and fimbriae 2/3 (FIM) were detected by ELISA, according to a method modified slightly from that described elsewhere [7, 13, 18, 22, 23]. Immunlon I microtiter plates (Dynatech) were coated with purified pertussis antigens (2 μg/mL, for IgG and IgA antibodies to PT and FHA; 3 μg/mL, for IgG and IgA antibodies to PRN; 1 μg/mL, for IgG antibody to FIM; and 2 μg/mL, for IgA antibody to FIM) (GSK) and were incubated overnight at 4°C. The next day, the plates were washed 5 times with washing buffer (PBS and 0.05% Tween 20), by use of an automatic ELISA plate washer (Tecan). Eight 2-fold serial dilutions of reference serum samples, control serum samples, and test serum samples were prepared by use of the automatic Matrix Impact 2 pipette and were added to each plate.

After being incubated for 3 h at room temperature, the plates were washed 5 times with buffer. Affinity-purified alkaline phosphatase–conjugated goat anti–human IgG or IgA antibody (Kirkegaard & Perry Laboratories) was added to each well, and the plates were incubated overnight at 4°C. On the third day, 100 μL of Sigma 104 paranitrophenyl phosphatase, with 1 mg/mL substrate solution, was added to each well. After incubation for 30 min at room temperature for color development, the plates were read by use of the Vmax kinetic microplate reader (Molecular Devices), at a 404-nm wavelength. ELISA units were computed by use of UnitCalc software (version 2.8), on the basis of the reference-line method [24, 25]. IgG ELISA units were determined by use of US reference pertussis antiserum (human) lots 3 and 4, and IgA antibody was standardized to lot 5 (US Food and Drug Administration [FDA]).

With FDA guidance and before assaying the present study's serum samples, a panel of 18 serum samples was used for external validation of the 4 IgG ELISAs. These 18 serum samples included 13 of the original 21 Center for Biologics Evaluation and Research standard serum samples that had been used to validate assays in a previous collaborative study that was conducted in the 1990s [26]. This panel was evaluated in triplicate on a single day, to assess intraassay variability, and was assayed 4 times over sequential days on different plates, to assess interassay variability. A similar validation assessment was conducted for the 4 IgA ELISAs, with a panel of 15 serum samples with IgA antibodies. Additional details about assay sensitivity, specificity, and reproducibility will be published elsewhere. The threshold of detection for IgG and IgA antibodies to each antigen was 2 ELISA units/mL; the limit of quantitation (LOQ) was 6 ELISA units/mL for IgG and IgA antibodies to PT and 8 ELISA units/mL for IgG and IgA antibodies to FHA, PRN,

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and FIM. The LOQ is the lower bound below which the precision of assay quantitation decreases.

Titers of *B. pertussis* agglutinin were determined by microagglutination, with *B. pertussis* strain BP 460 [23]. The lowest value was arbitrarily set at a 1:4 dilution.

Statistical analysis. Results of antibody assays were summarized by geometric mean titers (GMTs), with 95% confidence intervals that were based on the logarithmically transformed data. Levels below the threshold of detection were assigned a value of 1 (i.e., one-half of the threshold of detection). The mean titers for the study groups were compared at specified time points relative to immunization by 2-sample *t* test on the logarithmically transformed data. In addition, proportions were determined for (1) detectable antibody values at or above the LOQ and for (2) 2-fold increases, (3) 4-fold increases, and (4) 8-fold increases in antibody values at 1 month after immunization, relative to preimmunization antibody values. For the corresponding results, Spearman's rank correlations were computed at baseline and at 1 month after immunization.

Rates of antibody decay can be difficult to assess. First, a few immunized subjects had no increases in antibodies to PT, FHA, or PRN, and therefore there was no detectable antibody from which to assess decay. Second, some individuals had later increases in antibody—between 1 month (peak) and 18 months after immunization—presumably due to clinically unrecognized infections. Third, a few individuals had no apparent decay in antibody, suggesting that antibody values might be maintained by natural exposure to either *Bordetella pertussis*, other *Bordetella* species, or other organisms with similar antigens. Assessment of decay of antibodies to FIM was not possible, because it was not in the vaccine.

To assess the decay rates of specific antibodies, we restricted our analyses by excluding certain subjects. Specifically, we excluded the few nonresponders, subjects with persistently high titers, and subjects with interval increases to specific antigens.

The percent decay in GMTs of antibodies in the vaccinated cohort were computed for the following periods: 1–6 months after immunization, 6–12 months after immunization, 6–18 months after immunization, and 1–18 months after immunization. To compare the GMTs of the various antibodies for the overall period (1–18 months), 1-way analysis of variance (ANOVA) was used. To evaluate the pairwise differences, the Waller-Duncan *K*-ratio *t* test was used. To compare the antibody decay of different antibodies and of different subgroups of vaccinated subjects (e.g., those subjects who did or did not have preexisting detectable antibody) over time, repeated-measures ANOVA was used and the Greenhouse-Geisser correction was incorporated, to adjust the *P* values for the lack of circularity in the variance-covariance matrix [27]. Interaction between groups and time was the most important factor in the evaluation of the similarity of the decay rates.

In addition, we estimated, for each antibody, the length of time it takes for the GMT to fall below the LOQ. We assumed that the decay rate after 18 months would not appreciably change from that observed between 6 and 18 months after immunization. On the basis of that assumption, we computed the requisite length of time as $t = \log(l/m)/\log(1 - p)$, where t = time, m = GMT at 18 months for the given antibody, l = the LOQ of the assay, and p = the average decay rate for the 6–18-month period for the given antibody. To obtain the time from vaccination, we added 18 months to the calculated value. SEs of these estimates were computed by the delta method, on the basis of the first-order Taylor series approximation [28].

RESULTS

Preimmunization antibody values. As shown in tables 1 and 2, the GMTs of IgG and IgA antibodies in the preimmunization serum samples from ACP-vaccine recipients were similar to those in serum samples from control subjects, for all antigens studied ($P > .14$). Only 16% of all subjects (both vaccinees and control subjects) had values of IgA antibody to PT that were above the LOQ, and only 19% of all subjects had values of IgG antibody to PT that were above the LOQ; in contrast, 36%–63% of all subjects had values of IgA or IgG antibodies to FHA, PRN, or FIM that were above the LOQ. As will be detailed in a separate article concerning a study that included many more serum samples, there were no differences in preimmunization titers of antibody by age, sex, or geographic region. The presence of IgG antibody to PT that was above the LOQ did not correlate with the presence of IgA antibody to PT that was above the LOQ.

Postimmunization response. Tables 1 and 2, as well as figures 1 and 2, show the specific GMTs of antibodies after immunization. After immunization, the ACP-vaccine group experienced substantial increases in the GMTs of antibodies to all vaccine antigens (PT, FHA, and PRN). Specifically, the GMTs of IgG antibodies to PT, FHA, and PRN increased 17-, 28-, and 25-fold, respectively. As can be seen in table 1, 80%, 78%, and 82% of the vaccinated subjects had ⩾8-fold increases in titers of IgG antibodies to PT, FHA, and PRN, respectively, 1 month after immunization.

In contrast to the results for IgG antibodies, the results for IgA antibodies (table 2) were less marked. The GMTs of IgA antibodies to PT, FHA, and PRN increased 4-, 17-, and 15-fold, respectively, and only 27%, 68%, and 63% of the vaccinated subjects had ⩾8-fold increases of IgA antibodies to PT, FHA, and PRN, respectively.

Compared with the control subjects, at each postvaccination time point over the 18-month period, the vaccinees had significantly higher GMTs of IgG and IgA antibodies to all antigens ($P < .0001$, for all time points); 1 month after immunization, the GMTs of agglutinin antibodies also were significantly higher

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Table 1. Geometric mean titers (GMTs) (ELISA units/mL) of IgG antibodies to 4 *Bordetella pertussis* antigens, in vaccinated subjects and in nonvaccinated control subjects at selected time points.

Antibody, group	Before vaccination, GMT (95% CI) [% ⩾LOQ]	1 month after vaccination		6 months after vaccination, GMT (95% CI) [% ⩾LOQ]	12 months after vaccination, GMT (95% CI) [% ⩾LOQ]	18 months after vaccination, GMT (95% CI) [% ⩾LOQ]
		GMT (95% CI) [% ⩾LOQ]	Fold change, % (fold)[a]			
IgG to PT						
Vaccinated (n = 101)	2.20 (1.79–2.69) [21.8]	38.38 (31.79–46.33) [95.0]	99 (2×)	14.09 (11.25–17.64) [76.2]	8.84 (6.99–11.20) [70.7]	8.67 (6.85–10.97) [64.9]
	…	…	93 (4×)	…	…	…
	…	…	80 (8×)	…	…	…
Nonvaccinated (n = 99)	1.97 (1.61–2.40) [16.2]	2.05 (1.66–2.52) [16.2]	6 (2×)	1.98 (1.63–2.41) (17.2%)	1.96 (1.62–2.38) [16.2]	1.94 (1.59–2.36) [14.6]
	…	…	1 (4×)	…	…	…
	…	…	1 (8×)	…	…	…
IgG to FHA						
Vaccinated (n = 101)	12.68 (9.59–16.77) [59.4]	353.81 (296.58–422.08) [100]	96 (2×)	181.54 (147.64–223.22) [100]	125.59 (101.05–156.09) [98.0]	112.79 (91.17–139.54) [99.0]
	…	…	91 (4×)	…	…	…
	…	…	78 (8×)	…	…	…
Nonvaccinated (n = 99)	13.25 (10.16–17.27) [65.7]	13.27 (10.19–17.28) [62.2]	1 (2×)	12.82 (9.82–16.73) [62.6]	12.20 (9.33–15.95) [61.4]	13.91 (10.72–18.05) [65.7]
	…	…	1 (4×)	…	…	…
	…	…	1 (8×)	…	…	…
IgG to PRN						
Vaccinated (n = 101)	12.25 (9.43–15.90) [65.3]	304.31 (221.05–418.93) [99.0]	99 (2×)	169.72 (118.63–242.80) [94.0]	122.78 (85.19–176.96) [88.9]	117.17 (80.40–170.76) [89.4]
	…	…	93 (4×)	…	…	…
	…	…	82 (8×)	…	…	…
Nonvaccinated (n = 99)	11.39 (8.74–14.84) [59.6]	11.81 (9.10–15.32) [61.6]	6 (2×)	11.60 (8.96–15.02) [60.6]	11.17 (8.65–14.44) [60.6]	11.77 (9.07–15.27) [60.4]
	…	…	1 (4×)	…	…	…
	…	…	0 (8×)	…	…	…
IgG to FIM						
Vaccinated (n = 101)	4.94 (3.78–14.84) [40.6]	5.06 (3.85–6.64) [42.6]	3 (2×)	5.07 (3.86–6.65) [42.6]	5.09 (3.88–6.68) [40.4]	5.49 (4.16–7.24) [41.5]
	…	…	1 (4×)	…	…	…
	…	…	1 (8×)	…	…	…
Nonvaccinated (n = 99)	4.48 (3.43–5.84) [37.4]	4.45 (3.43–5.77) [36.4]	4 (2×)	4.35 (3.34–5.66) [37.4]	4.25 (3.27–5.53) [35.4]	4.29 (3.27–5.63) [35.5]
	…	…	0 (4×)	…	…	…
	…	…	0 (8×)	…	…	…

NOTE. CI, confidence interval; FHA, filamentous hemagglutinin; FIM, fimbriae 2/3; LOQ, limit of quantitation of assay; PRN, pertactin; PT, pertussis toxin.

[a] Fold change indicates the percentage of subjects with at least a 2-fold, 4-fold, or 8-fold increase in antibody level (before vaccination to 1 month after vaccination).

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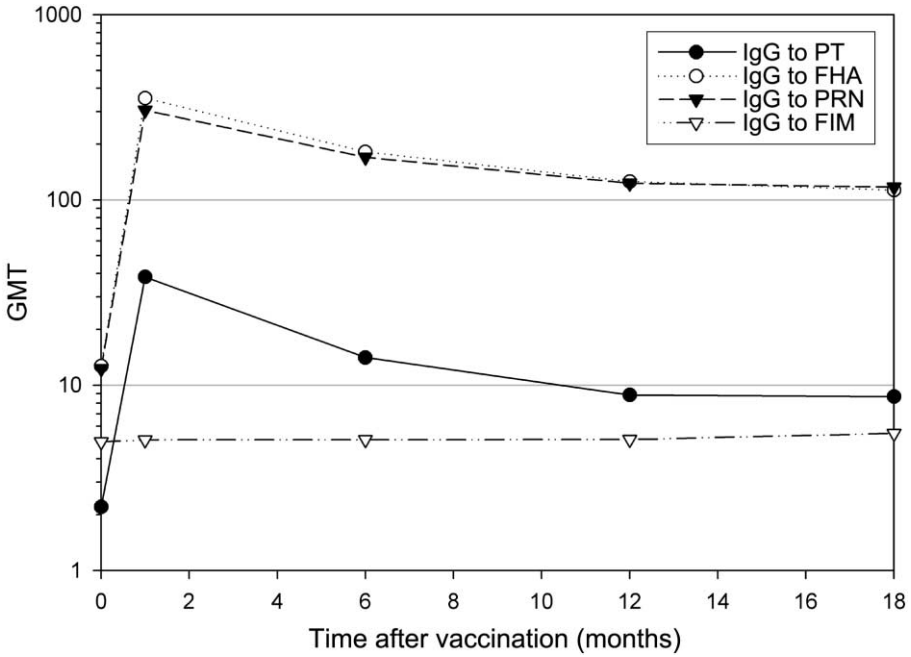

Figure 1. Geometric mean titers (GMTs) of IgG antibodies to pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3 (FIM), in 101 vaccinated subjects at 5 time points.

in the vaccinees, compared with those in the control subjects ($P = .004$), but only 9 (9%) of the vaccinees had ⩾4-fold increases in titers of antibody. No differences between the GMTs of IgG or IgA antibodies to FIM in the vaccinees and those in the control subjects were observed at any time point during the 18-month period ($P > .2$, for all instances).

Correlation of pre- and postimmunization antibody values. To evaluate the potential influence that preexisting antibody might have on subsequent vaccine response, a correlation analysis of preimmunization antibody values and postimmunization antibody values (1 month after immunization) was performed. For all antibodies, preimmunization values correlated directly with 1-month postimmunization values (IgG antibody to PT, $\rho = 0.56$; IgA antibody to PT, $\rho = 0.61$; IgG antibody to PRN, $\rho = 0.69$; IgA antibody to PRN, $\rho = 0.66$; IgG antibody to FHA, $\rho = 0.22$; IgA antibody to FHA, $\rho = 0.62$ [$P < .03$, for IgG antibody to FHA; $P < .0001$, for all others]). We also performed a qualitative analysis that compared the proportions of antibody values above and below the LOQ in the preimmunization serum samples with those in the postimmunization serum samples. The finding was similar to that of the quantitative analyses—preexisting antibody did not have an inhibitory effect on the response to immunization.

Rates of antibody decay in the serum samples from ACP-vaccinated subjects. The percent reductions in GMTs of IgG and IgA antibodies to PT, FHA, and PRN, over 5 intervals of time, are presented in table 3. There was significant antibody decay during each 6-month interval; between 1 and 18 months after immunization, the percent reduction for all antibodies ranged from 56.1% to 73.0%. IgG antibody to PT had the highest decay rate, whereas IgG antibody to PRN had the lowest decay rate ($P < .0001$, by ANOVA). IgG antibody to PT had a significantly higher percent reduction than did the other 5 antibodies assessed; IgA antibody to PT had a significantly lower decay rate. We attempted to understand potential variability in antibody decay by level of antibody. We examined 2 subgroups: (1) those subjects with the highest preimmunization antibody values (the highest 5%) and (2) those subjects with the highest postimmunization antibody values (the highest 5%). In neither of these subgroups did high antibody values influence decay rates, compared with the other subjects, excepting IgG antibodies to FHA and PRN; individuals who had detectable values of these antibodies before immunization had a significantly reduced rate of antibody decay, compared with individuals who had detectable values of other antibodies.

DISCUSSION

During the last half century, immunization of infants and children with pertussis vaccines has resulted in dramatic decreases in pertussis morbidity and mortality [1, 29, 30]. The reason the use of pertussis vaccines has been limited to children <7 years of age (with a few exceptions for outbreaks) is concern about vaccine reactogenicity and a general lack of recognition that *B. pertussis* cough illnesses in adolescents and adults were a problem. However, during the last 3 decades,

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Table 2. Geometric mean titers (GMTs) (ELISA units/mL) of IgA antibodies to 4 *Bordetella pertussis* antigens, in vaccinated subjects and in nonvaccinated control subjects at selected time periods.

Antibody, group	Before vaccination, GMT (95% CI) [% ⩾LOQ]	1 month after vaccination		6 months after vaccination, GMT (95% CI) [% ⩾LOQ]	12 months after vaccination, GMT (95% CI) [% ⩾LOQ]	18 months after vaccination, GMT (95% CI) [% ⩾LOQ]
		GMT (95% CI) [% ⩾LOQ]	Fold change, % (fold)[a]			
IgA to PT						
Vaccinated (n = 101)	2.36 (1.98–2.81) [18.8%]	9.07 (7.09–11.61) [69.3]	76 (2×)	5.63 (4.48–7.06) [48.5]	4.59 (3.69–5.72) [40.4]	4.11 (3.29–5.13) [36.2]
	…	…	51 (4×)	…	…	…
	…	…	27 (8×)	…	…	…
Nonvaccinated (n = 99)	1.97 (1.67–2.33) [13.1]	1.99 (1.65–2.39) [14.1]	5 (2×)	2.04 (1.70–2.44) [13.1]	2.02 (1.70–2.40) [13.1]	1.99 (1.67–2.36) [13.5]
	…	…	1 (4×)	…	…	…
	…	…	1 (8×)	…	…	…
IgA to FHA						
Vaccinated (n = 101)	5.48 (4.10–7.33) [39.6]	94.85 (68.10–132.11) [93.1]	95 (2×)	42.44 (30.24–59.56) [81.2]	30.69 (21.91–43.00) [77.8]	28.93 (20.78–40.28) [80.8]
	…	…	80 (4×)	…	…	…
	…	…	68 (8×)	…	…	…
Nonvaccinated (n = 99)	4.19 (3.18–5.52) [32.3]	4.19 (3.16–5.56) [31.3]	3 (2×)	4.15 (3.13–5.49) [31.3]	4.15 (3.16–5.44) [29.7]	4.38 (3.30–5.82) [32.3]
	…	…	1 (4×)	…	…	…
	…	…	1 (8×)	…	…	…
IgA to PRN						
Vaccinated (n = 101)	6.02 (4.59–7.90) [46.5]	91.95 (61.76–136.90) [87.1]	93 (2×)	47.45 (32.27–69.77) [79.2]	35.30 (24.09–51.72) [75.8]	33.40 (22.93–48.65) [76.6]
	…	…	80 (4×)	…	…	…
	…	…	63 (8×)	…	…	…
Nonvaccinated (n = 99)	5.35 (4.22–6.79) [41.4]	5.53 (4.26–7.18) [42.4]	5 (2×)	5.32 (4.09–6.91) [44.4]	5.33 (4.13–6.87) [44.4]	5.68 (4.43–7.28) [44.8]
	…	…	2 (4×)	…	…	…
	…	…	1 (8×)	…	…	…
IgA to FIM						
Vaccinated (n = 101)	8.17 (6.10–10.96) [52.5]	8.17 (6.12–10.93) [52.5]	3 (2×)	8.03 (5.97–10.81) [54.5]	7.96 (5.87–10.79) [53.5]	8.36 (6.11–11.45) [65.3]
	…	…	0 (4×)	…	…	…
	…	…	0 (8×)	…	…	…
Nonvaccinated (n = 99)	7.11 (5.12–9.87) [46.5]	7.07 (5.10–9.82) [46.5]	4 (2×)	6.75 (4.83–9.44) [45.4]	6.71 (4.81–9.36) [44.4]	6.74 (4.79–9.48) [42.7]
	…	…	2 (4×)	…	…	…
	…	…	1 (8×)	…	…	…

NOTE. CI, confidence interval; FHA, filamentous hemagglutinin; FIM, fimbriae 2/3; LOQ, limit of quantitation of assay; PRN, pertactin; PT, pertussis toxin.

[a] Fold change indicates the percentage of subjects with at least a 2-fold, 4-fold, or 8-fold increase in antibody level (before vaccination to 1 month after vaccination).

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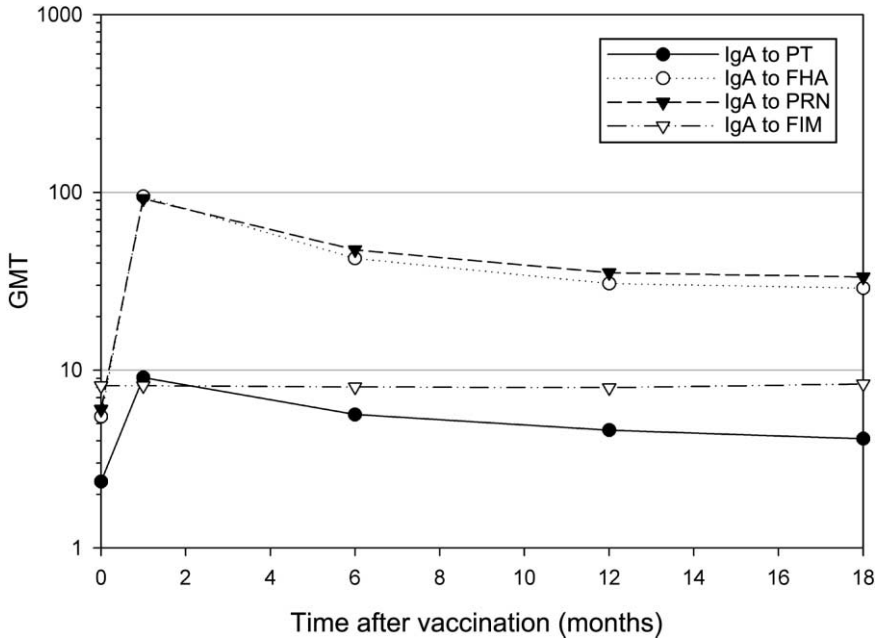

Figure 2. Geometric mean titers (GMTs) of IgA antibodies to pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3 (FIM), in 101 vaccinated subjects at 5 time points.

a large number of studies have noted the relative frequency and importance of *B. pertussis* infections in adolescents and adults [1–9, 11, 14–20, 22]. It has long been recognized that immunity that is induced by either vaccine or natural infection wanes over time.

We have found that, before immunization, preexisting values of IgG and IgA antibodies to PT that were above the LOQ were detectable in 16%–19% of the subjects, whereas preexisting values of IgG and IgA antibodies to FHA, PRN, or FIM that were above the LOQ were detectable in 36%–63% of the subjects (tables 1 and 2). Therefore, antibodies to PT may be more specific to the detection of recent exposure to pertussis, because antibodies to the other antigens seem to be sustained in a higher proportion of nonvaccinated subjects and may not be induced by exposure to *B. pertussis*, but rather to organisms that induce cross-reacting immunity [31–34].

In the present study, the response of IgG antibodies to the 3 vaccine antigens (PT, FHA, and PRN) is robust but is less than that noted previously by Van der Wielen et al. [35] and by Keitel et al. [21], both of whom used the same vaccine. In Van der Wielen et al.'s study [35], in which preimmunization GMTs of IgG antibodies are available, similar fold increases to the 3 antigens are noted. The GMTs of IgG antibodies to PT, FHA, and PRN found by Keitel et al. [21] were 70, 200, and 450 ELISA units/mL, respectively, and those found by Van der Wielen et al. [35] were 76, 750, and 588 ELISA units/mL, respectively. Of importance, the studies used different statistical methods to determine GMTs.

Even though the vaccine used in the present study had one-third of the concentrations of PT, FHA, and PRN that are found in the equivalent licensed pediatric formulation, the immune responses were similar to those found in a study in which the pediatric formulation was used [21]. For all antibodies evaluated—in particular those to PT—the IgG antibody responses were greater than the IgA antibody responses, but both responses did occur. Of the subjects in the present study, 80% had at least an 8-fold increase in IgG antibody to PT, compared with only 27% for IgA antibody to PT. Additional immunogenicity studies of ACP vaccines have been conducted in adolescents and adults [36–39].

Of interest is our finding of a response, in a majority of vaccinees, of IgA antibodies to all 3 vaccine antigens. As shown in table 2, 76%, 95%, and 93% of vaccinees had ⩾2-fold *increases* in titers of IgA antibodies to PT, FHA, and PRN, respectively. This result is in contrast to what is observed in children receiving primary immunization, who do not have an IgA antibody response [1, 32]. However, the IgA antibody response observed in the present study is likely explained by the fact that all adolescents and adults, whether vaccinated or not, have had previous *B. pertussis* infections [1, 7, 11, 13, 40].

We also evaluated the influence that preexisting antibody values might have on the immune response after immunization. A direct correlation between preimmunization titers and immune response was found, and no inhibition due to high preexisting antibody values was observed.

The IgG antibody decay patterns that are presented in table 1 and in figure 1 are quite similar to those observed both in other vaccine studies and after *B. pertussis* infection [21, 22, 38,

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Table 3. Percent reduction in geometric mean titers (GMTs) of IgG and IgA antibodies to pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin (PRN), over an 18-month period.

Antibody	No. of subjects[a]	1–6 months	6–12 months	6–18 months	12–18 months	1–18 months
IgG to PT	94	58.3 ± 22.0	34.6 ± 20.2	35.3 ± 30.0	0.4 ± 38.5	73.0 ± 17.8
IgG to FHA	100	42.2 ± 25.8	29.0 ± 14.9	35.2 ± 20.2	10.1 ± 21.2	61.3 ± 21.6
IgG to PRN	96	39.2 ± 26.6	22.9 ± 16.8	29.8 ± 30.3	9.5 ± 27.1	56.1 ± 23.6
IgA to PT	68	38.5 ± 24.2	18.7 ± 24.6	29.5 ± 28.3	9.2 ± 38.7	57.3 ± 21.2
IgA to FHA	93	52.8 ± 16.8	27.0 ± 14.1	37.2 ± 16.7	14.3 ± 14.5	69.6 ± 16.0
IgA to PRN	88	46.8 ± 16.6	24.3 ± 14.9	32.1 ± 18.3	10.6 ± 13.1	63.0 ± 17.5

NOTE. Data are % ± SE reduction in GMTs, except where noted.

[a] All subjects were immunized 1 month before the first serologic evaluation. The decay rates were determined for the given number of subjects; which subjects were excluded from this analysis is outlined in Subjects, Materials, and Methods.

41]. In the present trial, the GMT of IgG antibody to PT declined, from 1 month after immunization to 12 months after immunization, by 71%. In a previous study [21], after subjects were immunized with a vaccine similar to but more concentrated than the one used in the present trial, the GMT of IgG antibody to PT decayed by 79%. In 2 previous studies of *B. pertussis* illness, the decay in GMTs of IgG antibody to PT, from peak titers to those 16–18 months later, were 74% [22] and 81% [41].

In contrast to the rather marked decay in the GMT of IgG antibody to PT from 12 to 18 months after immunization, the slope of the decay in the GMT of IgG antibody to FHA is more gradual, as is the slope of the decay in GMT of IgG antibody to PRN. One can contrast antibody decay by comparing the levels of antibodies in immunized groups with those of unimmunized groups. Again, the findings of the present study are similar to those of both other vaccine trials and follow-up studies of natural infection [21, 22, 38, 41].

Of interest, the slope of the decay of antibodies to PT is different from that of antibodies to FHA and antibodies to PRN and is most likely due not to specific antigen-protein characteristics but to repeated exposure to similar FHA and PRN antigens during the follow-up period. Infections with other *Bordetella* species stimulate antibodies to *B. pertussis* FHA and PRN, as do infections with such pathogens as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae,* which have cross-reacting antigens.

To assess the potential long-term duration of antibodies, we assumed a decay rate equivalent to that for the last assessed time interval, 6–18 months. With the exception of the GMT of IgA antibody to PT, none of the GMTs had fallen below the LOQ by 18 months after infection (tables 3 and 4). As shown in table 4, the estimated duration of time that GMTs of antibodies will stay above the LOQ ranges from 1.5 to 9.1 years, and the estimated duration of time that the GMTs of antibodies will stay above the threshold of detection ranges from 3.6 to 13.0 years.

In adolescents and adults, the ACP vaccine used in the present study induces strong IgG and IgA antibody responses that decay in a predictable fashion such that, over a prolonged period of time, vaccinated subjects maintain significantly more antibody than do control subjects. Although it is current dogma that there are no serologic correlates of immunity related to *B. pertussis* infection, there is considerable evidence to the contrary [7, 42–46]. Recent data (in addition to data generated 50 years ago) indicate that low levels of IgG antibody to PRN in children are highly protective and that antibodies to PT and FIM also contribute to protection. If it is assumed that similar levels of antibody in adolescents and adults offer the same protection as do those observed in children, then the data in the present study can be used to suggest the frequency with which ACP-vaccine booster immunizations should be administered to adults, once adult-formulated vaccines become available in the United States. The slopes of the antibody decay (figures 1 and 2 and table 3) and the predicted duration of antibody levels (table 4) are reassuring in this regard; the decay in antibodies to PT seems to parallel susceptibility to repeated pertussis infections. If the presently recommended 10-year interval for DT booster immunizations is followed when an adolescent/adult

Table 4. Predicted duration that geometric mean titers (GMTs) of IgG and IgA antibodies to pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin (PRN) will stay above the assay's limit of quantitation and threshold of detection.

Antibody	Predicted duration ± SE, years	
	Above limit of quantitation	Above threshold of detection
IgG to PT	2.3 ± 0.9	4.9 ± 3.6
IgA to PT	<1.5	3.6 ± 2.4
IgG to FHA	7.6 ± 4.4	10.8 ± 6.7
IgA to FHA	4.3 ± 1.6	7.2 ± 3.3
IgG to PRN	9.1 ± 9.3	13.0 ± 14.0
IgA to PRN	5.2 ± 2.6	8.8 ± 5.1

NOTE. Data was extrapolated from the rate of antibody decay in subjects during the 6–18-month interval. See Subjects, Materials, and Methods for details on this and on cutoffs for the limit of quantitation and the threshold of detection.

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DTaP vaccine becomes available, one might expect that a substantial reduction in adolescent and adult pertussis will occur, as will a reduction in pertussis in unvaccinated infants. DTaP booster immunizations administered every 10 years may provide sustained protection and better interrupt the transmission of infections.

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