Natural History of Pertussis Antibody in the Infant and Effect on Vaccine Response

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To better characterize the transplacental transfer and persistence of pertussis antibodies and their role in the immune response to vaccine, concentrations of pertussis agglutinins and antibodies to lymphocytosis promoting factor (LPF) and filamentous hemagglutinin (FHA) were measured in three distinct groups of serum. Transplacental pertussis IgG antibody concentrations in newborns were found to be comparable to corresponding maternal concentrations and to decline with a half-life of ~6 weeks. By the age of 4 months, most infants had no detectable antibodies to LPF or FHA. Higher concentrations of maternally derived antibody to LPF were associated with a significantly weaker antibody response to conventional vaccine. In contrast, acellular vaccine stimulated superior antibody production, regardless of antecedent concentrations of antibody to LPF. The data support continuation of the current schedule of pertussis immunization and further efforts to develop an acellular vaccine for use in young infants.

Investigators have recently characterized many of the antigenic and biologically active components of *Bordetella pertussis*. The two components most extensively studied are the proteins lymphocytosis promoting factor (LPF) (also called pertussis toxin) and filamentous hemagglutinin (FHA) [1, 2]. These two antigens have been the major components of new acellular pertussis vaccines administered to several million Japanese children and to a small number of American children in clinical trials [3–6]. Case-control studies of acellular vaccine in Japan and efficacy studies in Sweden have shown that LPF and FHA are immunogenic and induce protection against pertussis disease [3, 7].

To understand better the transplacental transfer and persistence of antibody to these important immunogenic proteins, we have measured pertussis agglutinins (antibodies to surface determinants) and antibody concentrations to LPF and FHA in three distinct groups of serum. Group 1 comprised 34 sets of paired umbilical cord and maternal serum, collected to investigate maternal-fetal antibody transfer. Group 2 was serum collected from 50 infants immunized with either whole cell or acellular pertussis vaccines. Earlier studies had suggested that transplacental antibody blunted the immune response to conventional vaccine given below age 6 months [8–15]; this second group enabled evaluation of the effects of various concentrations of transplacental antibody on the response to these vaccines. Group 3, serial serum samples collected throughout the first 6 months of life from 17 children who had never been immunized against pertussis, permitted investigation of the natural decline of placentally acquired pertussis antibodies.

Materials and Methods

Specimens. Serum samples were obtained from three separate groups of lower and middle socioeconomic class children. In group 1, 34 pairs of mothers and newborn infants, umbilical cord specimens and maternal serum specimens were collected at term delivery at Nashville General Hospital in 1988. Group 2 comprised 50 infants who participated in a separate study in 1988. Specimens were collected at 2 months of age, before immunization with either conventional or acellular pertussis vaccine (with routine diphtheria and tetanus components), and at age 7 months, after three doses of vaccine. Group 3 consisted of 17 unimmunized infants who participated in an unpublished 1973 study (when pertussis was rare in the region). Four serum samples were collected from each infant during the first 6 months of life.

Samples were stored at −20°C. None of the infants were known to develop clinical pertussis.

Serologic assays. Antibodies to LPF and FHA were measured by ELISA using the standard methodology of Manclark et al. [16]. Purified LPF or FHA was used as a coating antigen (provided by Institut Merieux, Lyons, France). A standard reference human antiserum with an assigned value of 200 ELISA units/ml for each antibody was run in tandem with test serum on each plate. The lower limit of detection was 2 ELISA units for antibody to FHA and LPF. A parallel line bioassay method of analysis was used to compute unitage [16]. Results are expressed in ELISA units/ml.

The microagglutination assay used US lot 2 anti-pertussis rabbit serum with a titer of 1:51,200 as reference serum on each plate [16].

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Informed consent was obtained from the patients or their parents or guardians, and guidelines for human experimentation of the US Department of Health and Human Services and/or those of the authors' institution were followed in the conduct of the clinical research.

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The results were expressed as reciprocal dilutions with the lower limit of detection of 1:2.

Vaccines studied. Acellular pertussis vaccine, produced by Institut Merieux, consisted of LPF and FHA each independently purified and mixed together in equal proportions. The LPF was detoxified with glutaraldehyde and the vaccine was absorbed on aluminum hydroxide. The final product contained 12.5 μg LPF and FHA per 0.5 ml dose. The product contained no detectable agglutinogens [17]. Diphtheria and tetanus toxoids were combined with the acellular pertussis vaccine in quantities of 7.5 lime flocculation units (LF) and 5 LF, respectively, per 0.5 ml dose.

Conventional pertussis vaccine, obtained from a single lot produced by Connaught Laboratories (Swiftwater, PA), contained 4 units of pertussis vaccine, 6.7 LF diphtheria toxoid, and 5 LF tetanus toxoid per 0.5 ml dose.

Clinical trial study design, group 2. Fifty previously unimmunized infants were randomly assigned to receive either acellular or conventional vaccine in a blinded manner. Each infant received vaccine at 2, 4, and 6 months of age. Serum samples were obtained before each vaccination and 1 month after the last dose [17].

Statistical methods. Statistical calculations were performed on logarithmically transformed data [18]. For each appropriate group, geometric mean titers, standard errors, and 95% confidence intervals were calculated [19]. Within groups, mean geometric titer ratios (post-/pre- ratios) were calculated by dividing the sum of the individual geometric titer ratios by the number of subjects, then determining the antilogarithm; standard errors and 95% confidence intervals were calculated as above. Half-lives were compared using the z test on slopes; between-group comparisons were made using the t test for independent samples; within-group comparisons were made using the t test or the paired t test, as indicated [20].

Results

The first group of serum consisted of paired umbilical cord and maternal specimens (table 1). The mean titer of antibody to LPF in cord serum was 14.0 ELISA units, a value 2.9 times higher than found in maternal serum ($P = .057$). The mean titer of antibody to FHA in the infants was 26.8 units, which did not differ significantly from the value of 41.4 found in the mothers. Pertussis agglutinin antibody was essentially identical in the mothers and infants.

In the second group of serum, specimens obtained from 50 infants before immunization and after three immunizations with either a conventional (27 infants) or acellular (23 infants) vaccine, only the preimmunization titers were considered. As shown in table 1, the results were antibody to LPF, 2.6; antibody to FHA, 10.9; and pertussis agglutinins, 17.3. Mean preimmunization titers did not differ significantly between the two vaccine groups.

The third group of serum was from 17 infants who had never received pertussis immunization. Mean antibody to LPF and FHA and agglutinin titers declined progressively with age (table 1). The concordance between the mean titers for the group 2 infants and the group 3 infants of comparable age (visit 1) is striking, particularly in light of the 15-year interval between the two studies.

Figures 1–3 illustrate the natural history of pertussis antibodies in these unimmunized (or preimmunization) infants. The half-life of antibody to LPF was 36.3 days; antibody to FHA, 40.3 days; and pertussis agglutinins, 55.0 days (each pairwise $P > .05$).

Infants with relatively higher concentrations of antibody to LPF before immunization responded significantly less well to immunization with conventional vaccine than did infants with relatively lower preimmunization anti-LPF titers. This effect was consistent, regardless of the preimmunization titer

Table 1. Serologic assays of serum from maternal-newborn pairs (group 1), vaccine study infants before first immunization (group 2), and unimmunized infants (group 3).

	Group 1		Group 2	Group 3 (unimmunized) by visit			
Assay	Maternal	Cord	prevaccine	1	2	3	4
Antibody to LPF							
Mean age (days)	–	0	61 ± 12	55 ± 18	93 ± 22	125 ± 25	148 ± 21
No. serum samples	34	34	50	17	17	16	11
Geometric mean titer*	4.9	14.0	2.6	3.8	2.0	1.1	1.0
95% confidence interval	1.8–13.4	6.1–32.1	2.0–3.5	1.8–7.9	1.1–3.6	0.9–1.4	1.0–1.0
Antibody to FHA							
Mean age (days)	–	0	61 ± 12	55 ± 18	93 ± 22	124 ± 25	148 ± 21
No. serum samples	33	33	50	17	17	16	11
Geometric mean titer*	41.4	26.8	10.9	8.1	5.3	4.8	2.1
95% confidence interval	26.1–65.6	14.5–49.4	7.1–16.7	3.1–21.0	2.2–12.7	1.9–11.9	1.0–4.8
Agglutinins							
Mean age (days)	–	0	60 ± 14	58 ± 18	91 ± 20	123 ± 21	149 ± 20
No. sera	34	34	50	14	16	15	12
Geometric mean titer†	34.0	34.7	17.3	16.8	12.9	8.8	3.4
95% confidence interval	23.3–49.7	23.5–51.3	12–25	6.7–42.2	6.3–26.5	4.3–18.1	1.7–6.8

NOTE. LPF = lymphocytosis promoting factor, FHA = filamentous hemagglutinin.
* ELISA units/ml.
† Reciprocal end-point dilutions.

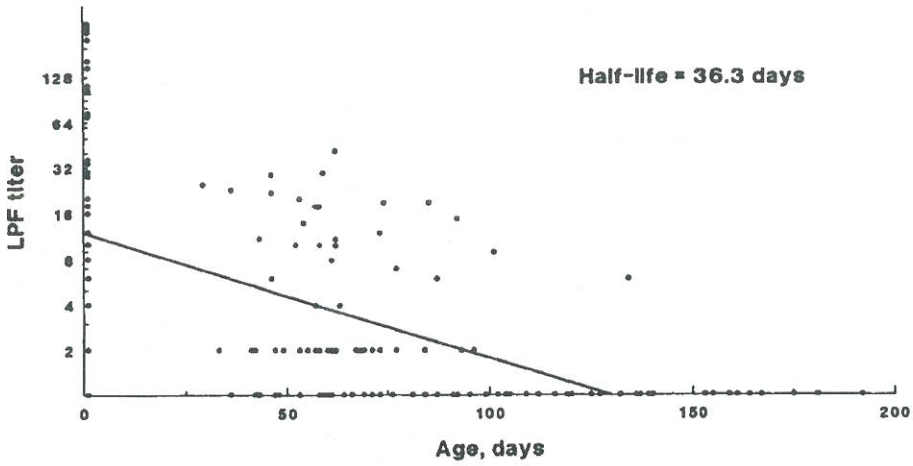

Figure 1. Antibody to lymphocytosis promoting factor (LPF) (ELISA units/ml) in serum samples from unimmunized infants in three study groups (for mean values see table 1). Regression line defines mean half-life.

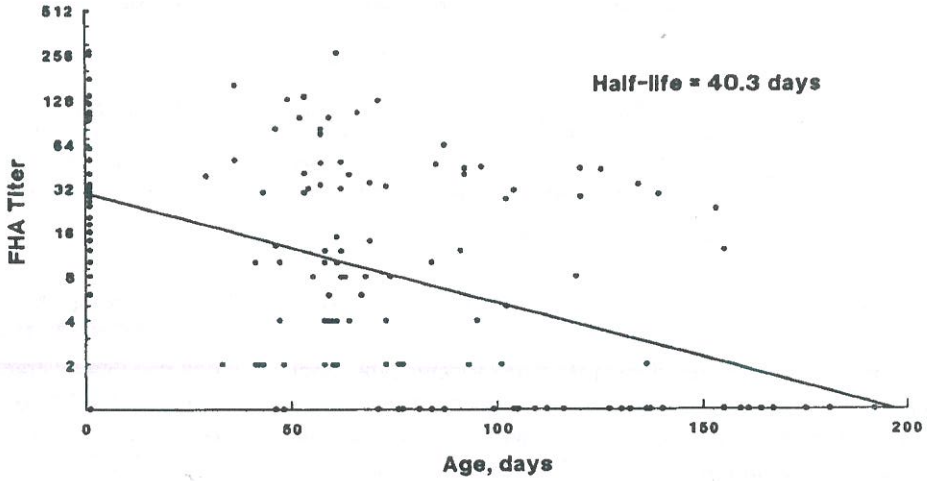

Figure 2. Antibody to filamentous hemagglutinin (FHA) (ELISA units/ml) in serum from unimmunized infants in three study groups (table 1 shows mean values). Regression line defines mean half-life.

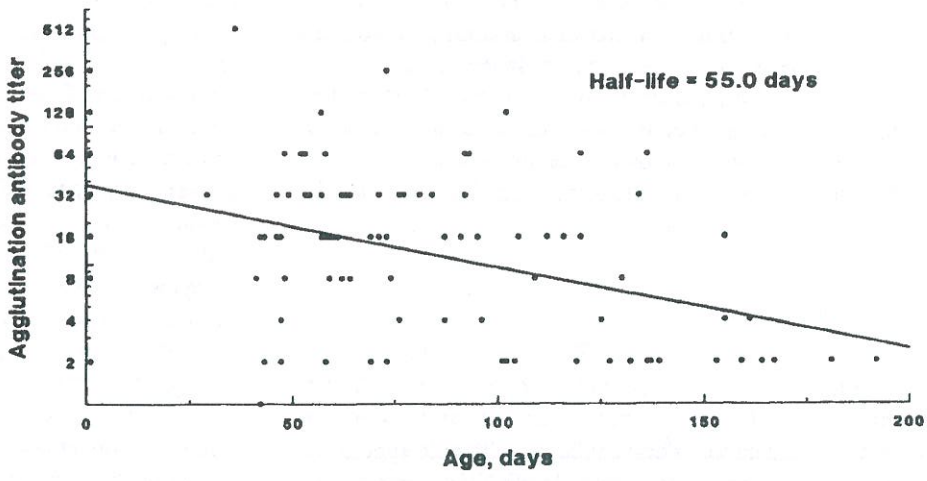

Figure 3. Pertussis agglutinins (reciprocal end-point dilutions) in serum from unimmunized infants in three study groups (table 1 shows mean values). Regression line defines mean half-life.

used to separate the infants into the low and high groups. For example, when the 22 infants receiving conventional vaccine whose preimmunization titer was ≤4 units were compared with the 5 whose baseline titer was >4 units, their postimmunization anti-LPF titers were 1.6 and 0.2 units, respectively ($P < .05$). Those with higher baseline anti-LPF titers also manifested a reduced response to FHA (22.9 vs. 11.7 units), but that difference was not statistically significant. These effects were not found among infants receiving acellular vaccine; for those infants, higher preimmunization anti-LPF titers cor-

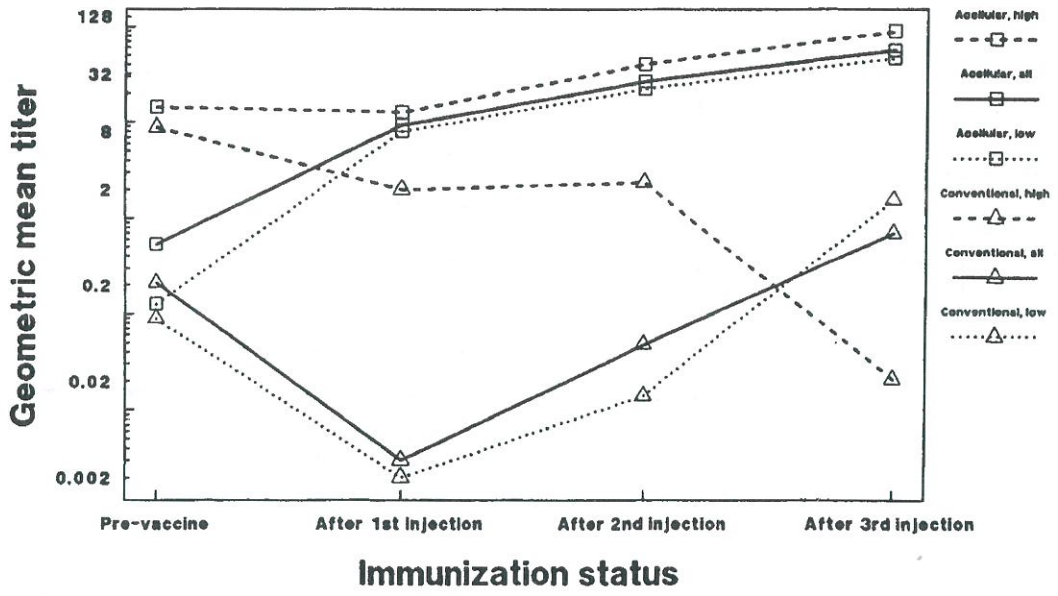

Figure 4. Antibody to lymphocytosis promoting factor (LPF) (ELISA units/ml) in infants receiving conventional and acellular vaccine, plotted by level of prevaccination LPF antibody. Lines marked with triangles show results with conventional vaccine; the squares, acellular vaccine. For each, the solid line shows overall results; the dashed line, results among infants with preimmunization LPF antibodies >4 ELISA units; the dotted line, results among infants with preimmunization titers of ≤4 ELISA units.

related with higher postimmunization anti-LPF and anti-FHA titers.

Figure 4 shows the relationships among vaccine type, level of transplacental antibody, and stage in the sequence of immunizations. For infants receiving the acellular vaccine, both those with low and those with high concentrations of preexisting antibodies to LPF established and maintained high antibody concentrations after the first dose of vaccine. Subsequent immunizations boosted their antibody. In contrast, infants receiving conventional vaccine in the presence of high concentrations of transplacental antibody showed a stable level of antibody to LPF after the first two doses of vaccine and a decline in antibody following the third dose.

A relatively higher level of transplacental antibody to FHA did not suppress vaccine response in either group.

Discussion

The importance of LPF and FHA as immunogens in protecting mice from challenge with *B. pertussis* is well documented [1–2]. Mice immunized with LPF survive both respiratory and intracerebral challenge with wild-type *B. pertussis* organisms; mice immunized with FHA survive respiratory challenge. In humans, acellular vaccines containing LPF and FHA administered to children in Japan have been 80%–90% effective in preventing disease in household contacts [21]. In Sweden, immunization of infants with two doses of purified LPF had an efficacy of 54% in preventing culture-positive pertussis [7]. Administration of two doses of a vaccine consisting of purified LPF and FHA resulted in a vaccine efficacy of 69% [7]. However, earlier studies in the same population had demonstrated an efficacy of 80% with whole cell vaccine [22]. Although most observers would agree that LPF and FHA likely play an important role in pertussis immunity, it is possible that other antigens, such as agglutinogens, may also be required to stimulate the production of optimally protective antibodies.

The principal motivation for development of acellular vaccines, of course, has not been dissatisfaction with the immunogenicity of the conventional vaccine; rather, it has been the relatively high rate of minor and serious adverse effects associated with the current US schedule of immunization with conventional vaccine (2, 4, and 6 months). However, as the Japanese experience has shown, these adverse effects can be reduced even with a conventional vaccine by substantially delaying the initiation of immunization [23]. An additional consideration is the concern that transplacental antibody may blunt the immune response to pertussis vaccine given below the age of 6 months [8–15]; delayed immunization avoids this concern. On the other hand, if immunization is delayed beyond the point at which transplacental antibodies decline below protective concentrations, then during that latter interval infants are left at risk for the morbidity and mortality associated with natural infection.

Therefore, we undertook studies to address three issues: (1) the measurement of antibodies in contemporary American women of child-bearing age and their transmission via the placenta to the newborn, (2) the natural history of anti-

body decay in children known to be unimmunized and uninfected by pertussis during the first 6 months of life, and (3) the effects of preexisting antibody on the response to two vaccines (conventional and acellular) that presented pertussis antigens in different forms.

We found that transplacental transfer produced concentrations of pertussis antibodies in the newborn comparable to or greater than those found in the mother. These results are consistent with prior studies that found that pertussis agglutinins, as well as antibodies to protein antigens of other microorganisms, appeared in cord blood of term infants in concentrations comparable to those found in the corresponding maternal serum [24–27].

Earlier studies showed that agglutinin titers had a half-life of 2 months and that little agglutinin antibody persisted by 6 months of age [8]. Using new methodology, we confirmed those findings and demonstrated comparable kinetics for antibodies to LPF and FHA. We found half-lives for all three pertussis antibodies to be ~6 weeks. The absolute quantities of antibody in both maternal and infant sera, however, were much lower than concentrations of antibody seen after primary vaccination or booster vaccine in older children [4, 17].

The particular susceptibility of small infants to life-threatening pertussis has been well documented [28, 29]. Although no correlates of protection with antibody concentrations to LPF, FHA, or agglutinins have been established, our data suggest that an infant not vaccinated during the first 6 months of life may be at increased risk for contracting pertussis. This supports the conclusions of Funkhouser et al. [30] that the adverse consequences of delayed pertussis vaccination (morbidity and mortality associated with increased natural infection) would greatly exceed the adverse consequences (vaccine reactions) associated with the current schedule of diptheria-tetanus-pertussis vaccination in the USA.

A suppressive effect of maternal pertussis antibody on the response of the infant to pertussis vaccination has been demonstrated by several investigators [8–15]. Studies in the 1940s of agglutinin antibody responses showed that the administration of conventional pertussis vaccine in the face of elevated antibodies was associated with reduced response [8–12]. In another report, immunoparalysis to pertussis agglutinin lasting 15 months was demonstrated when whole cell pertussis vaccine was administered during the first 24 h after birth [13]. It was recently shown that infants with relatively high antibody titers to LPF did not manifest a serologic response to LPF when immunized with whole cell pertussis vaccine [14, 15].

In our study, subjects with higher preimmunization concentrations of antibody to LPF manifested significantly lower postimmunization titers following conventional vaccine than those whose preimmunization concentrations of antibody had been lower. In contrast, the responses to acellular vaccines were independent of the preimmunization antibody titers. It is not known whether this improved response to acellular vaccines among those with higher antecedent anti-LPF titers is due to greater immunogenicity of LPF in the acellular product, the absence of other components of the whole cell vaccine that are lacking in the acellular product, or other as yet unidentified factors. Regardless, the lack of suppression of transplacental antibodies on the immune response to acellular vaccine enhances its attraction as a vaccine candidate for immunization in infants, beginning at age 2 months.

In summary, transplacental anti-pertussis IgG concentrations in infants are about equal to maternal concentrations. By 4 months postpartum, most infants have no measurable antibody to LPF or FHA. Higher concentrations of maternally derived antibody to LPF were associated with a weaker antibody response to conventional pertussis vaccine, but not to acellular vaccine.

We recommend that the current timing of the initial dose of pertussis vaccine be maintained so that endogenous pertussis antibody production may begin before the complete disappearance of maternally derived antibody. The finding that suppression of the humoral immune response to LPF by transplacental antibody did not occur following immunization with acellular vaccine, if verified, suggests an additional advantage of acellular vaccine when initiating immunization of infants at age 2 months.

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