Prevalence of Pertussis Antibodies in Maternal Delivery, Cord, and Infant Serum

C. Mary Healy,¹ Flor M. Munoz,¹² Marcia A. Rench,¹ Natasha B. Halasa,³ Kathryn M. Edwards,³ and Carol J. Baker¹,²
¹Section of Infectious Diseases, Departments of Pediatrics and ²Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas; ³Division of Infectious Disease, Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee

Background. Passively acquired maternal antibodies protect infants from many pathogens. With increasing reports of infant pertussis, we evaluated pertussis antibodies in maternal-infant paired sera from 1999–2000.

Methods. Antibodies to pertussis toxin (PT), filamentous hemagglutinin (FHA), and fimbrial proteins (FIM) were measured by validated IgG-specific enzyme-linked immunosorbant assay (ELISA) in 64 maternal–umbilical cord serum pairs and in 61 of 64 infant sera. Geometric mean concentrations (GMCs) of pertussis antibodies and cord:maternal GMC ratios were calculated.

Results. Mean maternal age and gestation were 29.7 years (range, 19–42) and 39.3 weeks (range, 35.6–40.9), and 81% of mothers were white. GMCs of maternal antibodies at delivery (ELISA units/mL) were 2.4 for PT, 6.9 for FHA, and 13 for FIM. Cord GMCs were 169%, 178%, and 157% of maternal delivery values for PT, FHA, and FIM, respectively, demonstrating active placental transfer (P<.001). Pertussis-specific IgG values for each antigen decayed to below the threshold of detection by age 2 months.

Conclusions. Despite efficient placental transfer, low maternal pertussis antibody levels and their rapid decay in infant sera leave infants with little humoral protection against pertussis. These data support the rationale for maternal or neonatal immunization, with acellular pertussis vaccines, to prevent life-threatening pertussis in early infancy.

Pertussis (or “whooping cough”), which is caused by the gram-negative pleomorphic bacillus Bordetella pertussis, is a severe, potentially life-threatening illness that caused substantial morbidity and mortality in the pre-vaccine era. Even allowing for past underestimation and underreporting of pertussis cases, many countries have experienced increasing pertussis disease rates and/or outbreaks during the past 2 decades, despite having effective immunization programs [1–5]. The reported annual incidence of pertussis in the United States has increased 3-fold since 1980, even though immunization rates for young children have been ≥80% [6, 7]. Adolescents and adults in whom vaccine-acquired immunity has waned are important sources of infection [8–14], which may be mild, atypical, and afflict as many as 80% of nonimmune household contacts who acquire pertussis from a primary case [5, 11, 15]. In contrast, very young infants are likely to present with atypical but severe illness [16]. During 1997–2000 in the United States, the highest pertussis attack rate (55.5 cases/100,000 population) occurred in infants <1 year of age (accounting for 29% of all reported cases), which is in great contrast to attack rates of 0.8–5.5 cases/100,000 population in other age groups. Complication rates were highest in infants <6 months of age—63% were hospitalized, 12% had pneumonia, 1% had seizures, 0.2% developed encephalopathy, and 0.8% died [6]. In 2000, each of the 17 pertussis-related deaths reported to the Centers for Disease Control and Prevention (CDC) occurred in US-born infants who had contracted pertussis at <4 months of age [17]. This continues a trend that was observed in the United States.
and Canada throughout the 1990s [16, 18].

Virulence factors associated with *B. pertussis* are either adhesion molecules (filamentous hemagglutinin [FHA], pertactin [PRN], BrkA, and fimbrial proteins [FIM]) or toxins (pertussis toxin [PT], tracheal cytotoxin, adenylate cyclase toxin, and dermonecrotic toxin). In animal models, active or passive immunization resulting in circulating antibodies to PT, to FHA, to PRN, and to FIM have been demonstrated to provide protective efficacy against lethal challenge [19–21]. Various pertussis antigens have been purified in sufficient quantities for inclusion in vaccines—PT and FHA are common to all acellular pertussis vaccines that are commercially available in the United States, and some formulations also contain PRN and FIM. The quantity of antibodies to vaccine antigens in serum can be used as a measure of adequate immune response, although what constitutes a “protective level” of antibody in serum has not been precisely defined.

Because the bulk of the morbidity and mortality associated with pertussis occurs in infants too young to have completed the primary diphtheria-tetanus toxoids–acellular pertussis (DTaP) vaccination series at 2, 4, and 6 months of age [6, 17, 18], we sought to determine both the level of maternal pertussis-specific antibodies transferred to infants and the kinetics of maternal-antibody decline in infants. A study conducted >10 years ago demonstrated low levels of maternal antibody [22]; however, a unique collection of archived maternal and infant serum samples permitted the determination, in a contemporary cohort of infants and their mothers, of the levels of IgG to *B. pertussis* antigens in serum.

**SUBJECTS, MATERIALS, AND METHODS**

**Study population.** Our subjects were women and their infants who had participated in 2 previous maternal immunization studies, conducted during 1999–2000, evaluating the safety and immunogenicity of group B streptococcal and respiratory syncytial virus vaccines [23, 24]. Participants eligible for enrollment in both studies were pregnant women who were 18–45 years of age and had a low risk for obstetrical complications. Exclusion criteria for these pregnant women included serious underlying disease, a history of febrile illness ≤72 h of enrollment, a severe reaction to any vaccine, an expected delivery before 37 weeks of gestation, multiple gestation, or antenatal detection of a major birth defect. Blood samples were obtained from each mother intrapartum (hereafter, “maternal delivery serum”), from the umbilical cord at the time of delivery (hereafter, “cord serum”), and from the infants at 2 months of age (hereafter, “infant serum”). Participants in the present study gave written, informed consent at the time of enrollment that unidentifiable blood samples could be used for future research on maternal and/or infant diseases. Our study was approved by the Institutional Review Board of Baylor College of Medicine (Houston, Texas).

**Laboratory methods.** Archived maternal delivery, cord, and infant serum samples were frozen, at −80°C, at Baylor College of Medicine. Aliquots of 100 μL of each serum sample, with identifiers removed, were shipped to Vanderbilt University School of Medicine (Nashville, Tennessee) for ELISAs, which had been validated in collaboration with both the US Food and Drug Administration (FDA) and the CDC. Pertussis-specific IgG to PT, to FHA, and to FIM were quantified by the methods described in detail by Meade et al. [25]. The same lot of standardized reference serum samples provided by the FDA was used both in the present study and in previous studies of levels of antibody from our laboratory [22, 26]. In brief, Immulon 2 microtiter plates (VWR International) were coated with either 1 μg/mL PT, 2 μg/mL FHA, or 0.5 μg/mL FIM. Serial dilutions of serum samples from mother-infant pairs were incubated for 2 h at 28°C, and an appropriate dilution of alkaline phosphatase–conjugated goat anti–human IgG was added. The reaction was developed and read at 405 nm. The threshold of detection for each assay was 2 ELISA units/mL, for PT; 3 ELISA units/mL, for FHA; and 5 ELISA units/mL, for FIM. For the purposes of statistical analysis, values less than the threshold of detection were considered to be one-half of the threshold of detection for that particular assay.

**Data analysis.** All data were analyzed at Baylor College of Medicine. Demographic and clinical data were analyzed by use of a standard statistical package (SPSS; version 11.5); dichotomous outcomes were compared by either the χ² test or Fisher’s exact test. Pertussis-specific IgG to PT, to FHA, and to FIM in serum were reported as geometric mean concentrations (GMCs), with 95% confidence intervals (CIs). For each antigen, comparisons of pertussis-specific IgG in maternal delivery serum and those in cord serum were performed by the Wilcoxon signed rank test. Placental transfer of antibody was defined, for each assay, as the ratio of cord GMC:maternal GMC. Plans were made to calculate, for each infant, the rate of the decline in pertussis-specific IgG over the course of 2 months by linear-regression analysis, on the condition that cord and infant levels proved to be sufficiently high.

**RESULTS**

**Study subjects.** In the 2 original immunization studies, 65 mother-infant pairs participated, and 64 of the mothers gave consent for the use of samples in future research. Sufficient quantities of serum were available for the testing of all 64 maternal delivery/cord serum-sample pairs and of 61 of the infant serum samples. Demographic and clinical data are summarized in table 1.

**Levels of pertussis-specific IgG in serum.** The GMCs, 95% CIs, and ranges of IgG to each pertussis antigen are summarized...
Table 1. Demographic and clinical data of mothers and their infants in the study population (n = 64).

| Maternal age, mean (range), years | 29.7 (19–42) |
| Ethnicity |  |
| White | 52 (81.2) |
| African American | 5 (7.8) |
| Hispanic | 4 (6.3) |
| Asian | 2 (3.1) |
| Other | 1 (1.6) |
| Sex of infants |  |
| Male | 25 (39.0) |
| Female | 39 (61.0) |
| Mode of delivery |  |
| Vaginal | 49 (76.6) |
| Cesarean | 15 (23.4) |
| Details of infants, mean (range) |  |
| Gestation, weeks | 39.3 (36–41) |
| Weight at birth, g | 3513 (2430–4802) |
| APGAR score at 1 min | 8 (1–10) |
| APGAR score at 5 min | 9 (7–10) |

Note. Data are no. (%), except where noted. APGAR, activity, pulse, grimace, appearance, and respiration.

Table 2. Levels of IgG to pertussis antigens, in maternal delivery, cord, and infant serum.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Geometric mean concentration (95% CI) [range], ELISA units/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maternal delivery serum&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pertussis toxin</td>
<td>2.4 (1.9–3.1) [1–33]</td>
</tr>
<tr>
<td>Filamentous hemagglutinin</td>
<td>6.9 (5–9.5) [1.5–137]</td>
</tr>
<tr>
<td>Fimbrial proteins</td>
<td>13.0 (9.2–18.5) [2.5–869]</td>
</tr>
</tbody>
</table>

Note. CI, confidence interval.

<sup>a</sup> Obtained from mothers intrapartum.
<sup>b</sup> Obtained from umbilical cords at the time of delivery.
<sup>c</sup> Obtained from infants at 2 months of age.

In Table 2, GMCs of IgG to PT, to FHA, and to FIM in maternal delivery serum were 2.4, 6.9, and 13.0 ELISA units/mL, respectively; GMCs of IgG to PT, to FHA, and to FIM in cord serum were 4.1, 12.3, and 20.4 ELISA units/mL, respectively; and GMCs of IgG to PT, to FHA, and to FIM in infant serum were 1.4, 3.0, and 5.8 ELISA units/mL, respectively. For each pertussis antigen, GMCs of IgG in cord serum were 1.6–1.8-fold higher than GMCs in maternal delivery serum (P < .001), and, for all but 1 mother-infant pair, IgG to PT, to FHA, and to FIM were greatest in cord serum (Figure 1A–C). Placental transfer of pertussis antibodies was 169%, for PT; 178%, for FHA; and 157%, for FIM. Levels of pertussis antibodies in cord and infant serum were too low to give meaningful estimates of rates of decline in pertussis-specific IgG.

**DISCUSSION**

Even in an era of high pertussis immunization rates, infant pertussis causes a substantial and well-recognized disease burden [7]. The recently reported increase in national pertussis disease rates has had particularly serious consequences for those infants too young to have completed the primary immunization series [6, 17, 18]. This trend also has been observed in Texas, the site of the present study, where, in 2002, 1240 pertussis cases (13% of the national total) were reported, which is more than double that reported in 2001 and the highest number of reported cases since 1964 [27]. In addition, 4 pertussis-related deaths occurred in Texan infants in 2002, each of whom were <3 months of age (L. J. Tabony, personal communication).

It has been suggested that the “resurgence” of pertussis [28] is due to greater awareness of the disease and improved diagnostic methods. In adolescents and adults, it certainly appears quite likely that a substantial portion of the increase in reported cases could be attributed to greater awareness. However, pediatricians are familiar with both the clinical presentation of infant pertussis and the increased risk of complications associated with it. They also have a greater degree of suspicion for pertussis when respiratory illnesses present in this age group, and diagnostic methods based on culture and on polymerase chain reaction have been available for some time. Finally, during the 1990s, the disease rate for pertussis remained relatively stable in children 1–4 years of age (in whom vaccine-induced immunity is high), while the rate increased in infants [6, 29, 30]. These facts suggest that the increased burden of disease reported in infants is real and is not due solely to improved reporting and/or diagnosis [6].

Our study examined, in samples obtained from a contemporary cohort, concentrations of pertussis-specific IgG in maternal delivery, cord, and infant serum. We have demonstrated that current maternal delivery levels of IgG to PT, to FHA, and to FIM are extremely low. A similar study conducted at the same laboratory 10 years ago assessed levels of antibody to PT and to FHA in maternal delivery and cord serum [22]. Although the levels found in this previous study were considered to be low, it is noteworthy that they were, nevertheless, 2- and 6-fold higher for PT and FHA, respectively, than were those found in serum samples from our cohort. Although archived serum samples from the 1990 study are no longer available—and thus the study cannot be repeated—the use of both a standardized methodology [25] and a consistent lot of positive
control serum samples make possible the comparison of concentrations of antibody over time [22, 26]. The seroprevalence of antibody to PT and to FHA in a cohort of 585 subjects who spanned a wide age range (1–65 years) and from whom samples were obtained during 1985–1990 also was reported from this laboratory [26]. Two peaks in antibody levels were found: the first at 4–6 years of age, corresponding with the fifth and last diphtheria-tetanus toxoids–pertussis vaccine (DTP) booster immunization, and the second at 13–17 years of age, probably representing natural pertussis infection. In that mixed-sex population, levels of pertussis-specific IgG in serum at 30 years of age, corresponding with the mean age of the mothers enrolled

Figure 1. Concentrations of IgG to pertussis toxin (A), to filamentous hemagglutinin (B), and to fimbrial proteins (C), in serum samples obtained from mothers intrapartum (●), from umbilical cords (■), and from infants at 2 months of age (▲). Each no. represents 1 mother-infant pair; note that the scales for the levels of IgG differ for each antigen.
in the present study, were significantly higher than those reported here. Our current data provide one possible explanation for the recent descriptions of maternal pertussis infection and transmission to infants [5, 9, 11, 31, 32].

Placental transfer to the infants who were at term gestation in our study of pertussis-specific IgG was >150%. Similar to other protein antigens (e.g., tetanus toxoid), active placental transfer of maternal pertussis-specific antibodies occurred, leading to substantially increased levels of pertussis-specific IgG available to the newborn infants [33–35]. However, the birth levels of pertussis-specific IgG declined rapidly, to negligible values by 2 months of age. Van Savage et al. [22] found similar rates of decline in antibody to PT and to FHA and calculated the half-life of maternal antibody to PT and to FHA to be 36 and 40 days, respectively. Nonetheless, the efficiency of placental transfer demonstrates the potential for neonatal and infant protection against pertussis when levels of maternal pertussis-specific antibody are elevated. Potentially, one way to confer maternal and infant protection might be to immunize mothers late during pregnancy. The concept of “boosting” maternal levels of antibody to pertussis is not a novel one; in the mid–20th century, it was explored via the active immunization of pregnant women with whole-cell pertussis vaccine during the third trimester of pregnancy [36–39]. No adverse maternal or neonatal effects were documented in these studies, and increased levels of antibody in both mother and infant were reported, as was enhanced in vitro killing of B. pertussis. However, the finding that preexisting high levels of pertussis antibody in infants suppressed the ultimate immune response to whole-cell DTP vaccines lessened enthusiasm for this approach [40–42]. Fortunately, this concern has proven unwarranted with the DTaP formulations that are now licensed for use in the United States and throughout much of the Western world. Published studies have not demonstrated suppression of DTaP vaccine responses in infants when levels of maternal antibody are high [22, 43, 44].

There are some limitations to our study. Our sample size was small and differed from the Texas birth cohort during the study period. Health statistics from the Texas Department of Health [45] indicate that, of infants born during 1999–2000, 45% were Hispanic, 40% were white, and 11% were African American; our cohort was predominantly white (81%). Furthermore, only 56% of Texas births during this time period were to women ≥25 years of age, compared with 84% in our population. The CDC has reported that a disproportionately high number of pertussis deaths occurs among Hispanic infants [17, 18] and that, outside of infancy, the peak age of natural pertussis infection is adolescence, with subsequent waning of levels of antibody [6, 26]. Differences between age and ethnicity in our population and those in the Texas birth cohort are, therefore, potential sources of bias in our study. In addition, the present study did not include the measurement of levels of antibodies to PRN in serum, which, according to some investigators, is a correlate of protection against mild and atypical pertussis disease [46, 47]. The reason for our exclusion of this antigen was that recent seroprevalence studies of pertussis antigens, conducted in a CDC-funded study, did not include this antigen for testing.

Notwithstanding considerable advances in pertussis immunization, the disease has remained a pernicious problem. Although current increasing disease rates may be due, in part, to improved reporting or diagnosis, infants <6 months of age who are too young to have completed their primary pertussis-immunization series are overrepresented in both morbidity and mortality figures [6, 16–18]. These infants remain vulnerable because levels of passively acquired maternal antibody appear to be too low to provide any significant degree of protection. The potential for protection of neonates and young infants exists by the provision, during pregnancy, of higher levels of pertussis-specific IgG. Our data support the rationale for the evaluation of maternal and neonatal immunization as possible approaches to prevent life-threatening pertussis in infants ≤4 months of age.

Acknowledgments

We thank Kathy Holland (Vanderbilt University Laboratory, Nashville, Tennessee), for performing the serological assays; Morven S. Edwards (Baylor College of Medicine, Houston, Texas), for helpful comments; and Robin Schroeder (Baylor College of Medicine), for assistance in preparing the manuscript.

References
