MAJOR ARTICLE

Kinetics of the Antibody Response to Tetanus-Diphtheria-Acellular Pertussis Vaccine in Women of Childbearing Age and Postpartum Women

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(See the Editorial Commentary by Munoz and Englund, on pages 893–6.)

Background. Because adolescents and adults act as a primary source of pertussis infection for infants, vaccination of mothers immediately postpartum is a potential strategy to reduce transmission (cocoon strategy). For this to be effective, high levels of antibodies must be achieved rapidly after vaccination. We sought to determine whether the antibody response to tetanus-diphtheria-acellular pertussis vaccine (Tdap) is sufficiently rapid to support the cocoon strategy.

Methods. Two sequential studies were performed. The first was a nonrandomized, open study of a 5-pertussis-component Tdap vaccine (tetanus toxoid, diphtheria toxoid, pertussis toxoid [PT], filamentous hemagglutinin [FHA], fimbriae types 2 and 3 [FIM], and pertactin [PRN]) given to women of childbearing age; the second was a randomized, open study of Tdap or no vaccine in postpartum women. Serum levels of immunoglobin (Ig) G and IgA against pertussis antigens, serum levels of IgG against diphtheria and tetanus, and breast milk levels of IgA against pertussis antigens were measured at various times after vaccination.

Results. In both studies, the antibody response was relatively rapid, with serum IgG and IgA levels beginning to increase noticeably by days 5–7 and approaching peak levels by day 14. Greater than 68% and 84.4% of IgG and IgA responders, respectively, achieved ≥90% of their maximum titer by day 14. The diphtheria and tetanus antibody kinetics followed a similar time course. Breast milk levels of IgA against PT, FHA, and FIM were first detectable at day 7, peaked by day 10, and then slowly decreased through day 28. Antibodies against PRN showed a similar response, although the peak occurred at day 14. There were no significant antibody responses in the control group.

Conclusions. Although the antibody response to a dose of Tdap in healthy nonpregnant women of childbearing age and postpartum women occurs by day 14 and is suggestive of an anamnestic immune response, it may not be sufficiently rapid to protect infants in the first weeks of life.

Pertussis (whooping cough) is a highly transmissible bacterial respiratory illness caused by *Bordetella pertussis* [1–5]. After the introduction and widespread use of pertussis vaccine in the 1940s, the incidence of reported pertussis decreased by >95% by the 1980s [6]. Over the past 25 years, however, pertussis rates have increased steadily, despite high childhood vaccination rates [1, 7]. Although much of this increase has been among adolescents and adults [7–10], pertussis incidence continues to increase among young infants, in whom much of the morbidity and all the mortality occur [1–3]. Canada and the United States, along with other developed countries, have reported a significant shift in morbidity toward infants <6 months of age [1–4, 11–14]. Parents

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(particularly mothers) and siblings have been cited as the most common sources of infection [2, 3, 5].

A variety of strategies have been proposed for protecting the newborn. Vaccinating the mother has the potential benefit of boosting maternal protection and, thus, decreasing the likelihood of maternal acquisition and transmission of infection to the susceptible newborn [5, 15, 16], the so-called cocooning effect. In a modeling study, cocooning was predicted to lead to a 9%–17% reduction in adult pertussis cases and a decrease of 70%, 65%, and 69% in cases among persons aged 0–3 months, 4–23 months, and 2–4 years, respectively [17]. Vaccinating women against pertussis during pregnancy or immediately postpartum may induce antibodies that can be transferred through the placenta to the fetus before birth or may induce specific secretory antibodies in breast milk that can be transferred through the milk to the newborn, providing passive protection against pertussis before the infant is protected by active vaccination.

These 2 sequential studies assessed whether the antibody response to adult-formulation tetanus-diphtheria-acellular pertussis vaccine (Tdap) is sufficiently rapid in healthy women of childbearing age to support the cocooning strategy. The kinetics of the antibody response to vaccination with Tdap was also assessed in postpartum women to determine whether a rapid increase in maternal serum levels is sufficient to achieve the transfer of antipertussis antibodies into breast milk.

METHODS

Study Design and Population

Two studies with different designs comprise this report. The first study was a nonrandomized, open study of the kinetics of the immune response to Tdap in women of child-bearing age. Healthy women 18–35 years of age were recruited in Halifax, Nova Scotia. Women were excluded if they had a history of a significant medical condition; were pregnant; were receiving immunosuppressive therapy; had a history of physician-diagnosed or laboratory-confirmed pertussis in the preceding 5 years; had received blood products or immunoglobulin within the preceding 3 months; had received a tetanus-, diphtheria-, or pertussis-containing vaccine in the preceding 5 years; or had received any vaccines within 2 weeks before enrollment. The second study was a randomized, open clinical trial of Tdap or no vaccine in postpartum women. In the second study, healthy women with an uncomplicated pregnancy were recruited in the third trimester from perinatal clinics of the IWK Health Centre and physicians' offices in the Halifax metropolitan area. Exclusion criteria for the second study were identical to the first, with the addition of any known fetal abnormalities. All participants provided written informed consent to participate; both studies were approved by the IWK Health Centre Research Ethics Board.

Vaccine

The licensed, adult-formulation Tdap (Adacel, Sanofi Pasteur) was used. Each 0.5-mL dose contained tetanus toxoid (5 limits of flocculation [Lf]), diphtheria toxoid (2 Lf), pertussis toxoid (PT; 2.5 μg), filamentous hemagglutinin (FHA; 5 μg), pertactin (PRN; 3 μg), fimbriae types 2 and 3 (FIM; 5 μg), aluminum phosphate (1.5 mg), and 0.6% of 2-phenoxyethanol.

Study Procedures

In the first study, after a medical history, history-directed physical examination, and negative result of a urine pregnancy test, 5 mL of blood was collected by venipuncture. A single 0.5-mL dose of Tdap was administered as an intramuscular injection into the deltoid muscle. Additional serum specimens were collected on days 1, 2, 3, 5, 7, 14, and 28 after vaccination. In the second study, women were recruited during the third trimester of pregnancy and were visited within 24 hours of delivery. At this visit, 1 mL colostrum (if present) and 5 mL of blood were collected, and participants were randomly allocated in a 4:1 ratio to receive a single 0.5-mL dose of Tdap or no vaccine. Additional colostrum or breast milk and blood samples were collected 7, 10, 14, and 28 days after vaccination.

Safety and Immunogenicity Monitoring

Serious adverse events and unanticipated contacts with a healthcare provider were recorded for the 28 days after vaccination. Serum samples were collected with a Vacutainer (BD-Canada); colostrum or breast milk was expressed and collected. Breast milk and serum samples were stored at −80°C until assayed. Antibodies were measured by enzyme immunoassays using standard methodology at the Canadian Center for Vaccinology in Halifax. Serum levels of immunoglobin (Ig) G against pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae types 2 and 3 (FIM) were reported as enzyme-linked immunosorbent assay (ELISA) units (EU) per milliliter with use of Food and Drug Administration–provided serum samples as the reference standard [18]. Serum tetanus and diphtheria IgG levels were reported as international units (IU) per milliliter. Breast milk levels of IgA against PT, FHA, PRN, and FIM were measured using enzyme immunoassay and were reported as a ratio of antigen-specific antibody to total breast milk IgA and expressed as EU per milligram total IgA.

Data Analysis and Statistical Considerations

The primary objective of the first study was to determine the rapidity of the serum antibody response after a single dose of Tdap and to determine the optimal time to measure antibody after vaccination for the second study in postpartum women. The primary objective of the second study was to determine whether antibodies against pertussis antigens are transferred into the breast milk in measurable quantities and to confirm that the antibody response to Tdap was of sufficient degree and

rapidity to provide protection to the mother during the postpartum period. The rapidity of the immune response was evaluated by measuring the time at which serum and breast milk antibody levels increased to greater than baseline levels and the proportion of responders who achieved 90% of the maximum antibody level in serum by day 14. Geometric mean antibody titers (GMTs) and exact 95% confidence intervals (CIs) were calculated for tetanus and diphtheria in serum samples and for PT, FHA, PRN, and FIM in serum and breast milk samples. The proportions (and 95% CIs) of participants achieving serum antitetanus and antidiphtheria titers ≥1.0 IU/mL were also calculated. For the pertussis antigens, the proportions (and 95% CIs) of participants achieving a ≥4-fold increase in serum antibody levels after vaccination relative to prevaccination levels were calculated.

No formal sample size calculation was performed for these descriptive and exploratory studies. A sample size of 30–50 participants per study was deemed to be sufficient to determine the kinetics of the immune response, based on the high rate of immune response to Tdap vaccine in this age group [19, 20].

RESULTS

Demographic Characteristics

Thirty healthy women 21–35.9 years of age (mean age, 27.9 years) were enrolled over 3 weeks in the first study. In the second study, 50 women 20–41 years of age were enrolled over 8 months and randomized in a 4:1 ratio to receive Tdap or no vaccine. The mean age of the 40 Tdap recipients was 29.7 years (range, 21–41 years), and the mean age of the 10 unvaccinated control women was 31.3 years (range, 20–37 years). All participants in the first study and 45 of 49 (91.8%) in the second study were white.

Serum Antibody

In the first study, healthy women of childbearing age developed detectable serum IgG to all pertussis antigens by days 5 (PT and FIM) to 7 (FHA and PRN), reaching a peak by day 14 (Figure 1). A similar serum IgA response was elicited to all pertussis antigens except anti-PT IgA (Figure 1*A*). The kinetics of the diphtheria and tetanus antitoxin responses were similar to those of the pertussis antigens, achieving levels of 1.0 IU/mL by approximately day 7 and peak levels by day 14 (Figure 1*E*).

In the second study, conducted in postpartum women, maternal serum IgG levels for tetanus, PT, FIM, and PRN were statistically significantly higher on day 7 ($P < .05$) compared with those for control participants. For diphtheria and FHA antibodies, Tdap recipients had significantly higher antibody levels than did control participants by day 10. Serum IgG levels for all antigens approached peak levels by day 10 and remained at these levels through day 28 (Figure 2). Serum IgA responses in Tdap recipients followed a similar pattern, with FHA, PRN, and FIM antibody levels being significantly higher in Tdap recipients than in control subjects on day 7 and PT antibody levels significantly higher in Tdap recipients than in control subjects by day 10. IgA approached peak levels by day 10; however, in Tdap recipients, IgA responses to all antigens began to noticeably decrease between days 14 and 28. Serum IgA responses against PT in the postpartum women were slightly increased (Figure 2*A*), in contrast to the somewhat elevated and nonincreasing levels in the women of childbearing age in the first study. There was no significant serum IgG or IgA response to any of the pertussis antigens in the unvaccinated control group. In Tdap recipients, diphtheria and tetanus antibody responses were similar to those of the pertussis antigens, with levels of 1.0 IU/mL reached by day 7 for tetanus and day 10 for diphtheria and remaining at these levels through day 28; there was no significant diphtheria and tetanus antitoxin response in the unvaccinated control group (Figure 2*E*).

In the first study, conducted in women of childbearing age, in those women who had an antibody response, levels of IgG to PRN, FIM, tetanus, and diphtheria increased more quickly than did levels of antibody to PT and FHA (92%, 89.7%, 96.7%, and 92.3% achieved 90% of their maximum IgG levels by day 14, compared with 68.0% and 74.1%, respectively) (Table 1). For IgA, 92.3%–100% of women achieved 90% of their maximum antibody levels by day 14. The proportion of postpartum women achieving 90% of their maximum antibody levels by day 14 was similar for all pertussis antibodies (Table 1).

More than 83.3% of participants in both studies achieved ≥4-fold IgG responses to all antigens (Table 1). The proportion of women with ≥4-fold serum IgA responses was lower than the proportion with similar IgG responses, particularly against PT and FHA (Table 1). All participants in study 1 and 95% of Tdap recipients in study 2 had antitetanus antibody levels >1.0 IU/mL; 86.7% and 67.5% of participants in studies 1 and 2, respectively, achieved antidiphtheria antibody levels >1.0 IU/mL.

Breast Milk Antibody

Breast milk IgA levels in Tdap recipients were already greater than those in unimmunized women by day 7 after vaccination. Breast milk levels of IgA against PT peaked on day 10, then slowly decreased through day 28. Breast milk levels of antibodies against FHA and FIM reached a plateau during days 10–14 and then slowly decreased. Breast milk levels of antibody against PRN peaked later on day 14 and then decreased by day 28 (Figure 3).

DISCUSSION

The increase in pertussis and pertussis-related deaths among young infants in many countries [1, 4, 16, 21–25] suggests that

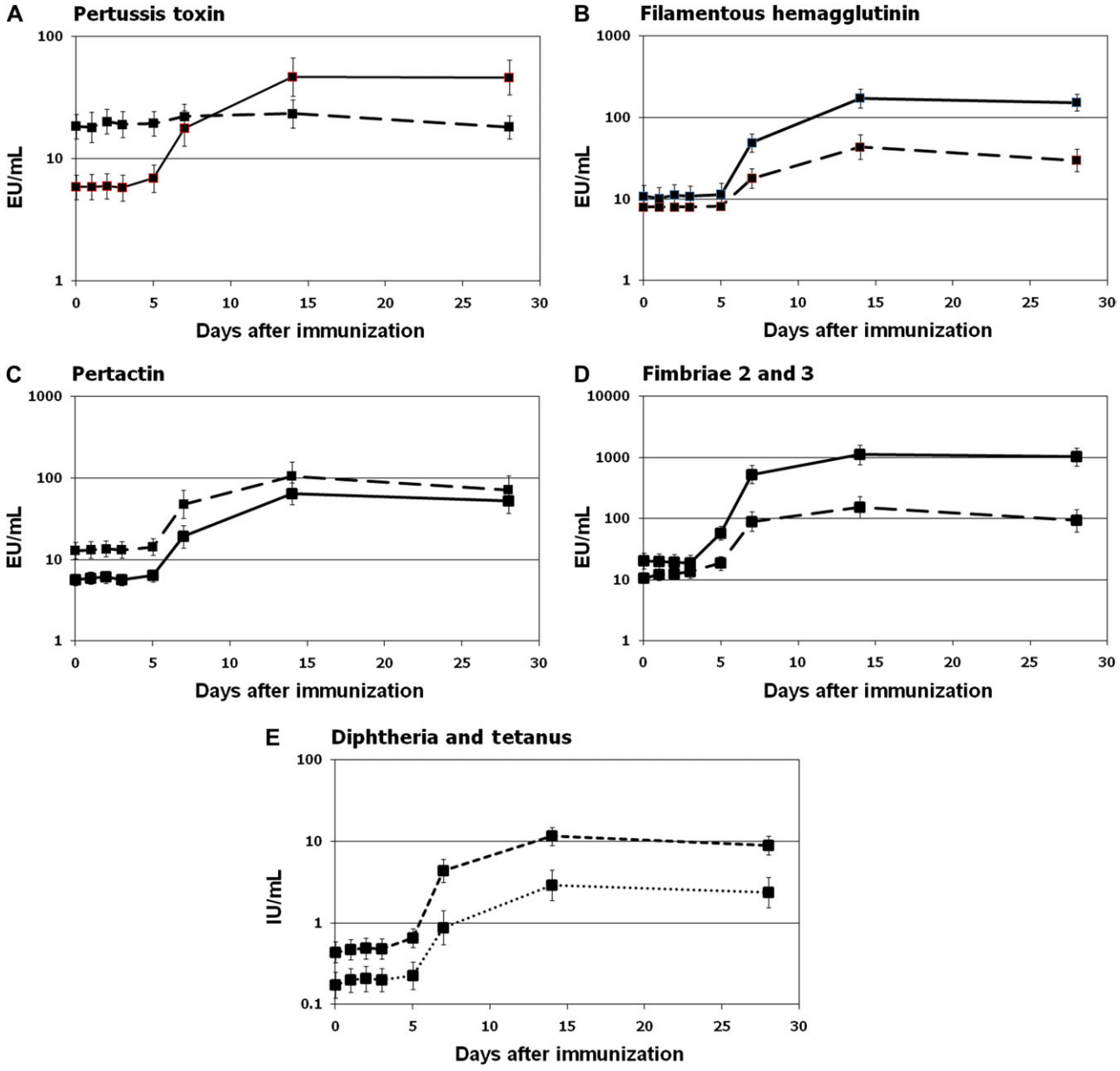

Figure 1. Geometric mean serum levels of immunoglobin (Ig) G (solid lines) and IgA (long dashed lines) antibody against pertussis toxoid (*A*), filamentous hemagglutinin (*B*), pertactin (*C*), and fimbriae 2 and 3 (*D*) before vaccination (day 0) and on days 1, 2, 3, 5, 7, 14, and 28 after vaccination with tetanus-diphtheria-acellular pertussis vaccine in women of child-bearing age. *E,* Antibody responses against diphtheria (dotted lines) and tetanus (dashed lines) toxoids.

additional strategies are required to protect infants before they complete their primary immunization series at 6 months of age. These studies explored the kinetics of the antibody response after Tdap vaccination in women of childbearing age and in postpartum women and show that postpartum pertussis vaccination efficiently increases maternal antibody levels and results in antipertussis antibodies in breast milk by 1–2 weeks after vaccination. Postpartum vaccination could therefore potentially provide temporary indirect protection (through the mother) and direct protection (through breast milk) for the infant from 2 weeks of age. However, the temporality of the response suggests that the infant would not be protected by this strategy until at least 2 weeks of age. Because of the 7–21-day incubation period of pertussis and the frequency of deaths in infants up to 6 weeks of age, it is clear that protection during this 2-week postpartum period is critical. Although postpartum vaccination could be a component of a public health strategy to protect infants, it would need to be supplemented by educational strategies about avoiding respiratory illness exposures and other infection control measures. The need for an immunization strategy that provides direct infant protection is clear.

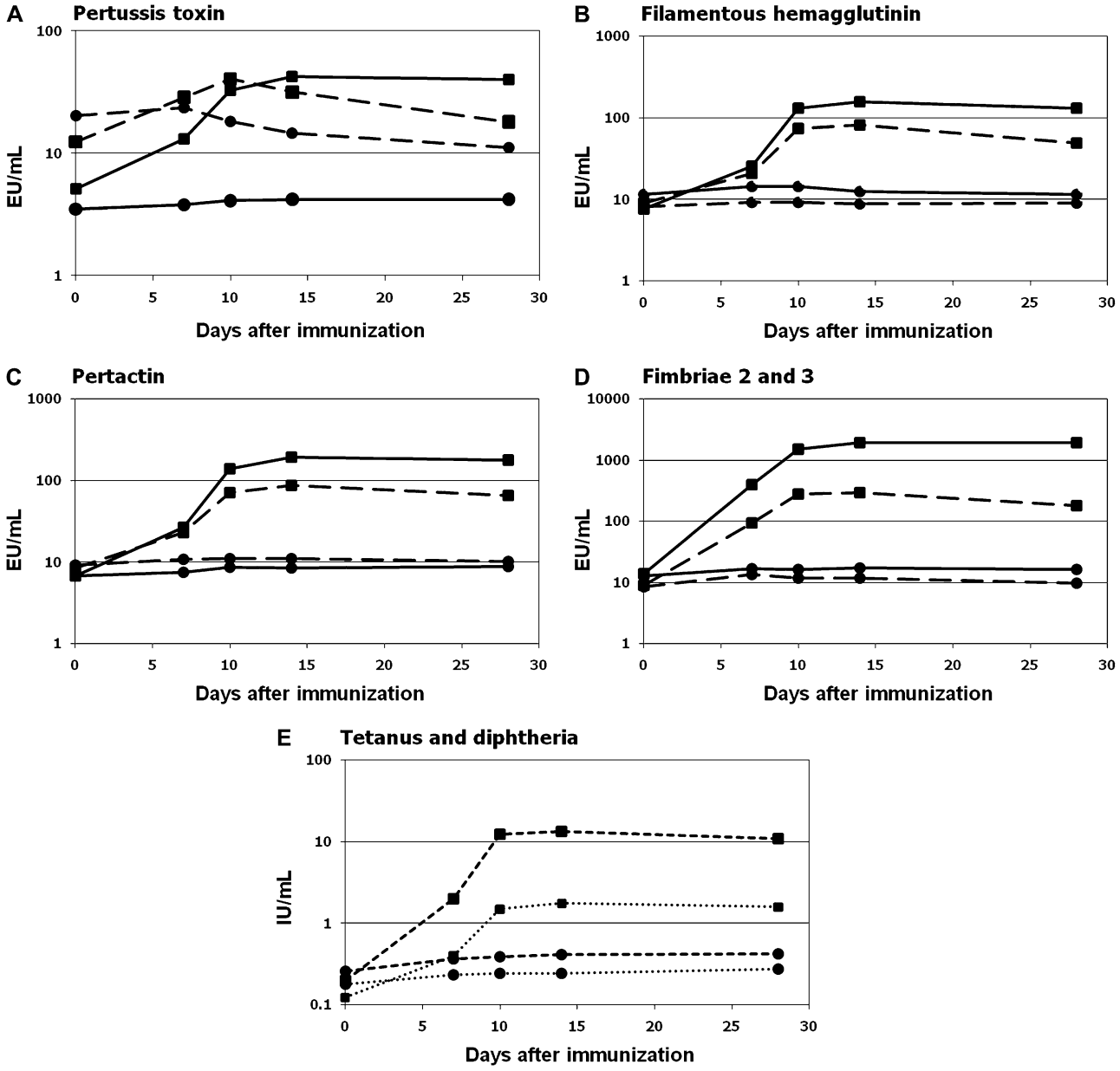

Figure 2. Geometric mean serum levels of immunoglobin (Ig) G (solid lines) and IgA (long dashed lines) antibody against pertussis toxoid (*A*), filamentous hemagglutinin (*B*), pertactin (*C*), and fimbriae 2 and 3 (*D*) in postpartum women before vaccination (day 0) and on days 7, 10, 14, and 28 after vaccination with Tdap (filled squares) and in unvaccinated (filled circles) postpartum women. *E,* Antibody responses against diphtheria (dotted line) and tetanus (dashed lines) toxoids in postpartum women vaccinated with Tdap (filled squares) and in unvaccinated postpartum women (filled circles).

The results of our 2 studies reveal that the increase to peak antibody levels requires 1–2 weeks. This is a significant finding because most women of childbearing age have low levels of antibodies to childhood diseases, including pertussis, and are thus unable to transfer substantial antibody to the fetus in the weeks before birth. Although pertussis antibodies are actively transported across the placenta [26, 27], low maternal levels would not be expected to provide protection to newborns. This absence of protection leaves open a window of susceptibility in the newborn, and we were unable to show a sufficiently rapid increase in antipertussis antibody levels in postpartum women during this vulnerable period.

Beginning the primary immunization series at 6 weeks of age, rather than at 8 weeks of age, and giving a neonatal dose of pertussis vaccine are being explored to reduce the incidence of pertussis in young infants. Two modeling studies have predicted that 10%–15% of cases in the first 2 months of age could be prevented by earlier initiation of the infant series [28, 29].

Table 1. Proportion of Participants Achieving Selected Threshold Antibody Levels After a Single Dose of Tetanus-Diphtheria-Acellular Pertussis Vaccine

Antigen	IgG class	Number (proportion; 95% confidence interval) of participants achieving 4-fold antibody response	Number (proportion; 95% confidence interval) of responders achieving >90% of maximal response by day 14	Number (proportion; 95% confidence interval) of participants reaching levels of 1.0 IU/mL
Healthy women of childbearing age				
Pertussis toxin	IgG	25/30 (83.3%; 65.3–94.4)	17/25 (68.0%; 46.5–85.1)	–
	IgA	3/30 (10.0%; 2.1–26.5)	3/3 (100%; 29.2–100)	–
Filamentous hemagglutinin	IgG	27/30 (90.0%; 73.5–97.9)	20/27 (74.1%; 53.7–88.9)	–
	IgA	19/30 (63.3%; 43.9–80.1)	18/19 (94.7%; 74.0–99.9)	–
Pertactin	IgG	25/30 (83.3%; 65.3–94.4)	23/25 (92.0%; 74.0–99.0)	–
	IgA	24/30 (80.0%; 61.4–92.3)	23/24 (95.8%; 78.9–99.9)	–
Fimbriae 2 and 3	IgG	29/30 (96.7%; 82.8–99.9)	26/29 (89.7%; 72.6–97.8)	–
	IgA	26/30 (86.7%; 69.3–96.2)	24/26 (92.3%; 74.9–99.1)	–
Tetanus	IgG	28/30 (93.3%; 77.9–99.2)	29/30 (96.7%; 82.8–99.9)	30/30 (100%; 88.4–100)
Diphtheria	IgG	28/30 (93.3%; 77.9–99.2)	24/26 (92.3%; 74.9–99.1)	26/30 (86.7%; 69.3–96.2)
Postpartum women[a]				
Pertussis toxin	IgG	34/39 (87.2%; 72.6–95.7)	29/34 (85.3%; 68.9–95.0)	–
	IgA	17/39 (43.6%; 27.8–60.4)	17/17 (100%; 80.5–100)	–
Filamentous hemagglutinin	IgG	39/39 (100%; 91.0–100)	35/39 (89.7%; 75.8–97.1)	–
	IgA	30/39 (76.9%; 60.7–88.9)	27/30 (90.0%; 73.5–97.9)	–
Pertactin	IgG	39/39 (100%; 91.0–100)	30/39 (76.9%; 60.7–88.9;)	–
	IgA	32/39 (82.1%; 66.5–92.5)	27/32 (84.4%; 67.2–94.7)	–
Fimbriae 2 and 3	IgG	38/39 (97.4; 86.5–99.9)	27/38 (71.1%; 54.1–84.6)	–
	IgA	37/39 (94.9%; 82.7–99.4)	36/37 (97.3%; 85.8–99.9)	–
Tetanus	IgG	39/39 (100%; 91.0–100)	36/38 (94.7%; 82.3–99.4)	38/40 (95%; 83.1–99.4)
Diphtheria	IgG	35/39 (89.7%; 75.8–97.1)	24/27 (88.9%; 70.8–97.6)	27/40 (67.5%; 50.9–81.4)

[a] None of the 10 unimmunized control women had a ≥4-fold increase in levels of antibody against pertussis toxin, filamentous hemagglutinin, pertactin, fimbriae 2 and 3, or tetanus and diphtheria toxins ($P \leq .009$ for all comparisons).

A neonatal dose of pertussis vaccine is being explored to improve the protection of young infants; however, clinical trials have shown mixed results [30–32].

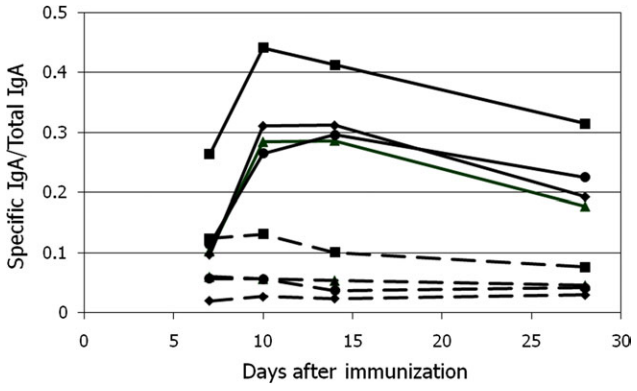

Figure 3. Geometric mean breast milk levels of immunoglobin (Ig) A antibody against pertussis toxoid (squares), filamentous hemagglutinin (triangles), pertactin (circles), and fimbriae 2 and 3 (diamonds) in postpartum women vaccinated with tetanus-diphtheria-acellular pertussis vaccine (solid lines) and in unvaccinated postpartum women (dashed lines).

The cocoon strategy has been proposed as a way to protect unvaccinated or incompletely vaccinated infants by selectively vaccinating new mothers and fathers [5, 15, 16]. This strategy protects the vaccinated group and, therefore, decreases the likelihood that these close contacts will bring infection into the household and transmit it to the infant; several studies have provided evidence that this strategy may be successful [33, 34]. The advantages include greater acceptability among women than vaccination during pregnancy, easier accessibility to other household members along with enhanced teaching opportunities, and the potential to combine postpartum immunization with a neonatal immunization program. The disadvantage, however, as shown by our data, is that the serum antibody response in postpartum women, although suggestive of an anamnestic immune response, may not be sufficiently rapid to protect infants in the first weeks of life. Although mucosal antibody protects newborn animals in the mouse [35] and pig [36] models of pertussis, there are no definitive data about the protective effect of breast milk antibodies against pertussis in humans [37]. These data suggest that the increase in pertussis-specific secretory IgA antibody levels in breast milk after

postpartum vaccination may not be sufficiently rapid to result in transfer of mucosal immunity to the infant via breast milk in the first 2 weeks of life. The advantages and disadvantages of all strategies need to be considered to determine the superiority of one approach over the other.

At present, the majority of infant exposures to pertussis are from adults [2, 5, 13, 15]. Despite recommendations from the US Advisory Committee on Immunization Practices and Canada's National Advisory Committee on Immunization that all adolescents and adults have a single booster with Tdap vaccine [21, 38], the recent large outbreaks of adult and adolescent pertussis indicate that this strategy has not yet been completely effective and underscore the urgency of identifying a strategy that will protect infants during this vulnerable period. Vaccinating preconceptually is unlikely to be successful because of the rapid decrease in maternal antibody titers that would be expected to occur during pregnancy [16]. As our studies have shown, vaccination during the immediate postpartum period ensures high maternal antibody levels; however, it takes 2 weeks for antibodies to reach maximum levels. Vaccinating women during the third trimester of pregnancy might protect the infant through placental transfer of maternal antibodies, resulting in high antibody levels in the infant at the time of birth and early appearance of mucosal antibodies in breast milk. This offers the possibility of protecting the infant from birth until immunity is achieved by active vaccination. Two studies exploring the role of maternal vaccination with Tdap are currently underway [39, 40].

Our studies had several limitations. The first study, performed in healthy women of childbearing age, was a nonrandomized pilot study with a small number of women (n = 30). In the second study of postpartum women, we were unable to collect and measure antibody levels in colostrums at baseline because some of the mothers experienced a delay in milk let-down and, in other women, the presence of nonspecific interference of colostrum in the enzyme immunoassay precluded antibody measurement. Of most significance, the mechanisms providing protection from pertussis are not well understood and there is no generally accepted correlate of immunity or seroprotective antibody concentration after pertussis or pertussis vaccination [41–43]. Protection from pertussis conferred by vaccination is at least partly dependent on cell-mediated immunity [44], and the relation of lower antibody concentrations and vaccine efficacy is unknown. Even with high levels of specific antibodies after vaccination, it is still not possible to define the precise levels of antibodies against a single antigen or a combination of antigens that correlate with protection. Therefore, although postpartum vaccination may be a viable protective strategy, the proof of whether this is true would best be demonstrated by a reduction in disease at the individual or population level.

In summary, these studies revealed that the serum and breast milk antibody response after vaccination with Tdap in postpartum women may not be sufficiently rapid to protect infants during the critical first 2 weeks of life. Although postpartum vaccination may be a part of the solution, other options, such as neonatal vaccination and vaccination of women in the middle to late third trimester of pregnancy may be required to fully protect infants during this vulnerable period.

Notes

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References

1. Vitek CR, Pascual FB, Baughman AL, Murphy TV. Increase in deaths from pertussis among young infants in the United States in the 1990s. Pediatr Infect Dis J **2003**; 22:628–34.
2. Bisgard KM, Pascual FB, Ehresmann KR, et al. Infant pertussis: who was the source? Pediatr Infect Dis J **2004**; 23:985–9.
3. Halperin SA, Wang EE, Law B, et al. Epidemiological features of pertussis in hospitalized patients in Canada, 1991–1997: report of the Immunization Monitoring Program–Active (IMPACT). Clin Infect Dis **1999**; 28:238–43.
4. Mikelova LK, Halperin SA, Scheifele D, Smith D, Ford-Jones E, et al. Predictors of death in infants hospitalized with pertussis: a case-control study of 16 pertussis deaths in Canada. J Pediatr **2003**; 143:576–81.
5. Wendelboe AM, Njamkepo E, Bourillon A, et al. Transmission of *Bordetella pertussis* to young infants. Pediatr Infect Dis J **2007**; 26: 293–9.
6. Halperin SA. Canadian experience with implementation of an acellular pertussis vaccine booster-dose program in adolescents: Implications for the United States. Pediatr Infect Dis J **2005**; 24(6 Suppl):S141–S146.
7. Guris D, Strebel P, Bardenheir B, et al. Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 1990–1996. Clin Infect Dis **1999**; 28:230–7.
8. De Serres G, Shadmani R, Duvall B, et al. Morbidity of pertussis in adolescents and adults. J Infect Dis **2000**; 182:174–9.
9. Skowronski DM, De Serres G, MacDonald D, et al. The changing age and seasonal profile of pertussis in Canada. J Infect Dis **2002**; 185:1448–53.
10. Centers for Disease Control and Prevention. Pertussis—United States, 2001–2003. MMWR Morb Mortal Wkly Rep **2005**; 54:1283–6.
11. Baron S, Njamkepo E, Grimple E, et al. Epidemiology of pertussis in French hospitals in 1993 and 1994: thirty years after a routine use of vaccination. Pediatr Infect Dis J **1998**; 17:412–8.
12. He Q, Viljanen MK, Arvilommi H, Aittanen B, Mertsola J. Whooping cough caused by *Bordetella pertussis* and *Bordetella parapertussis* in an immunized population. JAMA **1998**; 280:635–7.
13. Ranganathan S, Tasker R, Booy R, Habibi P, Nadel S, Britto J. Pertussis is increasing in unimmunized infants; is a change in policy needed? Arch Dis Child **1999**; 80:297–9.
14. Elliott E, McIntyre P, Ridley G, et al. National study of infants hospitalized with pertussis in the acellular vaccine era. Pediatr Infect Dis J **2004**; 23:246–52.

15. McIntyre P, Wood N. Pertussis in early infancy: disease burden and preventive strategies. Curr Opin Infect Dis **2009**; 22:215–23.
16. Mooi FR, de Greef SC. The case for maternal vaccination against pertussis. Lancet Infect Dis **2007**; 7:614–24.
17. Van Rie A, Hethcote HW. Adolescent and adult pertussis vaccination: computer simulations of five new strategies. Vaccine **2004**; 22:3154–65.
18. Halperin S. Serologic and molecular tools for diagnosing *Bordetella pertussis*. In: Detrick B, Hamilton R, Folds J, eds. Manual of Molecular and Clinical Laboratory Immunology. 7th ed. Washington, DC: American Society for Microbiology Press; **2006**. p.540–6.
19. Halperin SA, Smith B, Russell M, et al. Adult formulation of a five-component acellular pertussis vaccine combined with diphtheria and tetanus toxoids and inactivated poliovirus vaccine is safe and immunogenic in adolescents and adults. Pediatr Infect Dis J **2000**; 19:276–83.
20. Halperin SA, Smith B, Russell M, et al. An adult formulation of a five-component acellular pertussis vaccine combined with diphtheria and tetanus toxoids is safe and immunogenic in adolescents and adults. Vaccine **2000**; 18:1312–9.
21. Broder KR, Cortese MM, Iskander JK, et al. Preventing tetanus, diphtheria, and pertussis among adolescents: Use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccines. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep **2006**; 55:1–34.
22. Tan T, Trindade E, Skowronski D. Epidemiology of pertussis. Pediatr Infect Dis J **2005**; 24(5 Suppl):S10–S18.
23. Ulloa-Gutierrez R, Avila-Aguero ML. Pertussis in Latin America: current situation and future vaccination challenges. Expert Rev Vaccines **2008**; 7:1569–80.
24. Roehr B. Whooping cough outbreak hits several US states. BMJ **2010**; 341:c4627.
25. Lawrence R. Whooping cough on the rise in Saskatchewan. Moose Jaw Times Herald 30 September **2010**. Available at: http://www.mjtimes.sk.ca/News/Local/2010-09-30/article-1809662/Whooping-Cough-on-the-rise-in-Saskatchewan/1. Accessed 29 December 2010.
26. Van Savage J, Decker MD, Edwards KM, Sell SH, Karzon DT. Natural history of pertussis antibody in the infant and effect on vaccine response. J Infect Dis **1990**; 161:487–92.
27. Healy CM, Munoz FM, Rench MA, Halasa NB, Edwards KM, Baker CJ. Prevalence of pertussis antibodies in maternal delivery, cord, and infant serum. J Infect Dis **2004**; 190:335–40.
28. Foxwell AR, McIntyre P, Quinn H, Roper K, Clements MS. Estimated impact of first vaccine dose at 6 versus 8 weeks in Australia. Pediatr Infect Dis J **2011**; 30:161–3.
29. Shinall MC, Peters TR, Zhu Y, Chen Q, Poehling KA. Potential impact of acceleration of the pertussis vaccine primary series for infants. Pediatrics **2008**; 122:1021–6.
30. Halasa NB, O'Shea A, Shi JR, LaFleur BJ, Edwards KM. Poor immune responses to a birth dose of diphtheria, tetanus, and acellular pertussis vaccine. J Pediatr **2008**; 153:327–32.
31. Wood N, McIntyre P, Marshall H, Roberton D. Acellular pertussis vaccine at birth and one month induces antibody responses by two months of age. Pediatr Infect Dis J **2010**; 29:209–15.
32. Knuf M, Schmitt HJ, Wolter J, et al. Neonatal vaccination with an acellular pertussis vaccine accelerates the acquisition of pertussis antibodies in infants. J Pediatr **2008**; 152:655–60.
33. Coudeville L, van Rie A, Andre P. Adult pertussis vaccination strategies and their impact on pertussis in the United States: Evaluation of routine and targeted (cocoon) strategies. Epidemiol Infect **2008**; 136: 604–20.
34. Kandola K, Lea A, White W, Santos M. A comparison of pertussis rates in the Northwest Territories: pre- and post-acellular pertussis vaccine introduction in children and adolescents. Can J Infect Dis Med Microbiol **2005**; 16:271–4.
35. Oda M, Izumiya K, Sato Y, Hirayama M. Transplacental and transcolostral immunity to pertussis in a mouse model using acellular pertussis vaccine. J Infect Dis **1983**; 148:138–45.
36. Elahi S, Buchanan RM, Babiuk LA, Gerdts V. Maternal immunity provides protection against pertussis in newborn piglets. Infect Immun **2006**; 74:2619–27.
37. Pisacane A, Graziano L, Zona G, et al. Breast feeding and acute lower respiratory infection. Acta Paediatr **1994**; 83:714–8.
38. National Advisory Committee on Immunization (NACI). Statement on the prevention of pertussis in adolescents and adults. Can Commun Dis Rep **2003**; 29:1–9.
39. Safety and immunogenicity of Tdap vaccine in healthy pregnant women, safety in their neonates, and effect of maternal immunization on infant immune responses to dTap Vaccine. Available at: www.clinicaltrials.gov. Sponsor: National Institute of Allergy and Infectious Diseases (NIAID). ClinicalTrials.gov Identifier: NCT00707148. Accessed 29 December 2010.
40. Immunization of women with diphtheria and tetanus toxoids combined with acellular pertussis (Tdap) during the mid third trimester of pregnancy: An evaluation of the potential for immunological protection for the neonate. Available at: www.clinicaltrials.gov. (Sponsor: Dalhousie University; collaborators: IWK Health Centre, Sanofi Pasteur.). ClinicalTrials.gov Identifier: NCT00553228. Accessed 29 December 2010.
41. Cherry JD, Gornbein J, Heininger U, Stehr K. Search for serologic correlates of immunity to *Bordetella pertussis* cough illnesses. Vaccine **1998**; 16:1901–6.
42. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. Vaccine **1998**; 16:1907–16.
43. Hewlett EL, Halperin SA. Serological correlates of immunity to *Bordetella pertussis*. Vaccine **1998**; 16:1899–900.
44. Meyer CU, Zepp F, Decker M, et al. Cellular immunity in adolescents and adults following acellular pertussis vaccine administration. Clin Vaccine Immunol **2007**; 14:288–92.