Title: Immune Responses to Pertussis Antigens in Infants and Toddlers after Immunization with Multicomponent Acellular Pertussis Vaccine

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Abstract

Given the resurgence of pertussis despite high vaccination rates with the diphtheria-
tetanus-acellular pertussis (DTaP) vaccine, a better understanding of vaccine-induced
immune responses to *Bordetella pertussis* (*B. pertussis*) is needed. We investigated the
antibody, cell-mediated, and cytokine responses to *B. pertussis* antigens in children who
received the primary vaccination series (at 2, 4, and 6 months) and first booster
vaccination (at 15-18 months) with 5-component acellular pertussis (aP) vaccine. The
majority of subjects demonstrated 4-fold increase in antibody titer to all four pertussis
antigens (pertussis toxin[PT], pertactin[PRN], filamentous hemagglutinin[FHA], and
fimbriae[FIM]) following the primary series and booster vaccination. Following the
primary vaccine series, the majority of subjects (52-67%) mounted a positive T-cell
proliferative response (stimulation index ≥3) to PT and PRN antigens, while few
subjects (7-12%) mounted positive proliferative responses to FHA and FIM. One month
after booster vaccination (age 16-19 months), our study revealed significant increase in
IFN-γ production in response to PT and FIM antigens, significant increase in IL-2
production with PT, FHA, and PRN antigens, and lack of significant IL-4 secretion with
any of the antigens. While previous reports documented a mixed Th1/Th2 or Th2-
skewed response to DTaP vaccine in children, our data suggest that following the first
DTaP booster, children aged 16-19 months have a cytokine profile consistent with a
Th1 response, which is known to be essential for clearance of pertussis infection. To
better define aP-induced immune responses following the booster vaccine, further
studies are needed to assess cytokine responses pre- and post-booster in DTaP
recipients.
Introduction

*Bordetella pertussis* (B. pertussis) is a significant cause of morbidity and mortality worldwide, especially in young children (1, 2). Following widespread use of the whole cell pertussis (wP) vaccines in the 1940s, the incidence of pertussis in the United States, which had previously exceeded 200,000 cases annually, declined dramatically (3, 4). Due to the relatively high rate of adverse local and systemic effects associated with wP vaccine, safer acellular pertussis (aP) vaccines replaced wP in the United States in the mid-1990s (5). The aP vaccine contains fewer antigens than the wP vaccine and lacks the reactogenic endotoxin (6). In the 1980s, the incidence of pertussis began to increase again, and despite high immunization rates with the aP vaccine, over 48,000 cases of pertussis were reported in the United States by 2012, the highest incidence since 1955 (3). While infants continue to be at greatest risk for pertussis infection, there is evidence that the rate of pertussis has been increasing among adolescents and adults (3, 7). Moreover, older individuals play an important role in transmission of pertussis to young infants, who are at the highest risk of complications and mortality from infection (3, 7).

There are several theories that may explain the rise in cases of pertussis, including improved methods of detection such as polymerase chain reaction assays, vaccine-induced antigenic variation of the *B. pertussis* organism, poor or waning immunity conferred by the current aP vaccines, or some combination of these factors (1, 6, 8, 9). Given the resurgence in pertussis cases despite high vaccination rates, it is important to better characterize the mechanisms of immune protection against *B. pertussis*. While many human and mouse studies have examined the immune response...
to *B. pertussis* infection and vaccination, the exact mechanism of immunity and
correlates of protection remain unclear (1, 10).

Several studies provide evidence for the roles of both antibody and cell-mediated
immune response to *B. pertussis* (11-14) in prevention of disease and infection. Many
human and mouse studies have investigated the relative contribution of Th1 (type 1
helper T cell) and Th2 (type 2 helper T cell) responses to pertussis infection and to both
wP and aP vaccines (15-22). Most studies have found that natural pertussis infection
and wP vaccine induce a predominantly Th1 response to pertussis antigens (15, 17-20).
While the majority of studies with aP vaccine describe a mixed Th1/Th2 or Th2-
predominant response (2, 12, 16, 18, 20), a few studies document a Th1-predominant
response (21, 22). Furthermore, there are varying results regarding which of the *B.
pertussis* antigens are the most or least effective at inducing antibody and cell-mediated
responses and cytokine production. In order to gain better understanding of vaccine-
induced immune responses, our study aimed to investigate the antibody, cell-mediated,
and cytokine responses to *B. pertussis* antigens in children under two years of age who
received their primary series and first booster vaccination with multicomponent aP
vaccine.

**Materials and Methods**

**Study design overview.** This was an open-label, single-arm, single-center, descriptive
study designed to assess antibody and cell-mediated immune responses (CMI) to
pertussis antigens in children who received the primary aP vaccine series and first
booster. Subjects were enrolled from a local pediatric practice in Madison, Tennessee,
from September 2005 to February 2006. This study was approved by both the Western Institutional Review Board and Vanderbilt Institutional Review Board. Informed consent was obtained from the parents or legal guardians of all participants.

Vaccine. The vaccine, manufactured by Sanofi Pasteur Limited (Pentacel™), is a combination product. Each 0.5 mL dose contains 15Lf diphtheria toxoid, 5Lf tetanus toxoid, and the following acellular pertussis antigens: 20 µg detoxified pertussis toxin (PT), 20 µg filamentous hemagglutinin (FHA), 3 µg pertactin (PRN), and 5 µg fimbriae types 2 and 3 (FIM). It also includes inactivated poliovirus (IPV; 40 D-antigen units [DU] of type 1, 8 DU of type 2, and 32 DU of type 3 poliovirus) and 10µg purified capsular polysaccharide of Haemophilus influenzae type b (Hib) covalently bound to 24 µg of tetanus toxoid. The vaccine contains 1.5 mg aluminum phosphate as the adjuvant.

Study population. Criteria for enrollment were healthy infants 42-84 days of age, at least 37 weeks gestational age at delivery, free of obvious health problems as determined by medical history and clinical examination before entering the study, no known or suspected impairment of immunologic function, and no contraindication to the vaccine. Subjects with recent fever (less than 72 hours prior to visit) or with a history of having received the diphtheria-tetanus-aP (DTaP), DTwP, Hib-conjugate, poliovirus, or pneumococcal-conjugate vaccines prior to enrollment were excluded.
Study schedule and procedures. Four doses of the combination study vaccine were administered at approximately 2, 4, 6, and 15-18 months of age. Other standard vaccines were given as recommended by the American Academy of Pediatrics (AAP) (Table 1). Blood samples were collected for analysis of *B. pertussis* antigen-specific antibody and T cell proliferation prior to the first dose of Pentacel™ (2 months of age, pre-primary series), one month after the third dose (7 months, post-primary series), prior to the fourth dose (15-18 months, pre-booster) and one month after the fourth dose (16-19 months, post-booster). Antigen-specific cytokine production was only measured on post-booster samples.

Serum antibody determinations. Sera were tested for anti-PT, -FHA, -PRN, and -FIM immunoglobulin G (IgG) titers by enzyme-linked immunosorbent assay (ELISA) using a standardized protocol as previously described (23-25). ELISA units were assigned based on the U.S. Food and Drug Administration human reference pertussis antisera (Lot 3). Immulon 2 plates were coated with optimized antigen concentrations of 1 µg/mL of PT, 2 µg/mL of FHA, 2 µg/mL of PRN, or 0.5 µg/mL of FIM. The lower limits of detection of IgG antibody were 2 EU/mL for PT, 3 EU/mL for FHA, and 5 EU/mL for FIM. Sequential serum samples from each subject were run simultaneously in the same assay. Serial two-fold dilutions starting at 1:60 were performed for each sample. ELISA calculations were performed based on a log linear model using SoftMax Pro (Molecular Devices). Seroconversion to *B. pertussis* antigen was defined as a four-fold increase in antibody titers from baseline (pre-primary series dose).
Lymphocyte sample processing. Blood samples were collected, held at room temperature, and processed within 2 hours. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Sigma-Aldrich) density gradient centrifugation, and resuspended at a concentration of \(1 \times 10^7\) cells/mL in freezing medium containing 90% fetal bovine serum (Invitrogen Life Technologies) and 10% dimethyl sulfoxide (DMSO). The cells were then transferred to cryogenic vials, placed in Nalgene Cryofreezing containers (Nalge Nunc International), and stored at \(-80^\circ\)C. Frozen specimens were transferred to a liquid nitrogen freezer and stored in the vapor phase. At the time of analysis, cryopreserved cells were thawed in a 37°C water bath, incubated with 20 \(\mu\)g/mL DNase (Roche), and washed twice. Viability was determined by trypan blue exclusion. The lymphocyte proliferation and cytokine assays were performed from January to May 2007.

Lymphocyte proliferation assay. The capacity of PBMCs to respond to pertussis antigens was measured by antigen-specific proliferation. Triplicate cultures of PBMC suspension (2 x \(10^5\) cells/well) were incubated with 1 \(\mu\)g of heat-inactivated PT/ml, 10 \(\mu\)g FHA/ml, 10 \(\mu\)g FIM/ml, or 10 \(\mu\)g PRN/ml. PBMC cultures without stimulus were used as a negative control, and PBMC cultures stimulated with pokeweed mitogen (1 \(\mu\)g/ml) were used as a positive control. After 5-day incubation at 37°C in an atmosphere of 5% CO\(_2\), 3H-thymidine (0.5 \(\mu\)Ci/well) was added to the cultures, and incorporated radioactivity was measured by scintillation counting 16 hours later. The results were expressed as mean counts per minute (cpm) from triplicate wells. In the event that there were insufficient PBMCs to test all the pertussis antigens, we tested individual antigens.
in the following order of priority: PT, FIM, PRN, and then FHA. Only samples with viability of 70% or greater were used for analysis (average viability was 89.8%). A T cell proliferative response was defined to be positive when the *B. pertussis* antigen-induced proliferation was at least three-fold higher than the spontaneous proliferation (stimulation index, SI $\geq 3$). As a basic quality criterion, the proliferative response of PBMCs to pokeweed mitogen (PWM) had to be at least 10-fold higher than spontaneous proliferation (SI $\geq 10$).

**Cytokine assays.** Different CD4$^+$ helper T cell subsets have distinct patterns of cytokine secretion (26). Th1 cells produce IL-2 as well as IFN-γ, which regulates the cell-mediated immune response and helps macrophages kill engulfed intracellular pathogens (20, 22, 27). Th2 cells secrete IL-4, IL-5, and IL-13, which mediate defense against helminths and drive allergic disease (26, 27). TNF-α has been measured as a Th1 cytokine (28) and as a control cytokine (29) which is produced by unstimulated, nonproliferating cells. IL-10 is secreted by various cells including Th1, Th2, regulatory T cells, and innate immune cells (26, 30). For this study, we considered significant IL-2 and IFN-γ production to be consistent with a Th1 response, and IL-4 and IL-5 to represent a Th2 response. IL-10 and TNF-α were not categorized as a predominately Th1 or Th2 response.

To determine antigen-specific cytokine production, a 100 µl aliquot of supernatant from cultures established for the measurement of lymphocyte proliferation was harvested at 48 hours, quick frozen, and stored at -80°C. Cultures were replenished with media and kept until day 6 as described above. Cytokine bead arrays
(CBA) detecting IFN-γ, TNF-α, IL-2, IL-4, IL-5, and IL-10 (Becton Dickinson) were performed according to the manufacturer’s instructions (31); intra- and interassay imprecision of the CBA for all six cytokines has been previously evaluated by Tarnok et al (32). The detection limits were 3.3 pg/mL for IFN-γ, 1.1 pg/mL for TNF-α, 1.1 pg/mL for IL-2, 1.3 pg/mL for IL-4, 1.3 pg/mL for IL-5, and 1.4 pg/mL for IL-10.

**Statistical analysis**

The geometric mean titers (GMT) of anti-PT, anti-FHA, anti-PRN, and anti-FIM antibody concentrations were calculated using the log-transformation of concentrations and taking the anti-log of the mean of these transformed values. The magnitude of change in the T-cell proliferative response between the pre- and post-primary series, and between the post-primary and pre-booster time points was compared using Wilcoxon signed rank test. The difference in cytokine production in response to pertussis antigen and in non-stimulated cultures was compared by Wilcoxon signed rank test. For all analyses, a two-tailed $P$ value $\leq 0.05$ was considered to be significant. All analyses were performed using R version 3.0.2 (www.r-project.org).

**Results**

**Study population.** A total of 50 subjects were enrolled and evaluated for antibody, proliferative, and cytokine responses. The mean age at enrollment was 62 days (43 to 84 days), mean birth weight 3.4 kg, mean gestational age 39 weeks; 54% were female, 74% were categorized as white, 16% as black, and 10% as “other” race. One subject
dropped out of the study following the third vaccine, prior to the collection of the post-
primary vaccination sample, because the family relocated.

**Humoral response.** Table 2 demonstrates serum antibody concentration against the
four *B. pertussis* antigens (PT, FHA, PRN, FIM) and the percentages of subjects with
seroconversion at pre-primary, post-primary, pre-booster, and post-booster sampling
points. The trend in antibody response to each *B. pertussis* antigen at each sampling
point is presented in Figure 1. One month following the third vaccine dose (post-primary
series, at approximately 7 months of age), the GMT to each pertussis antigen was
significantly higher than the pre-vaccination levels. At the pre-booster sampling point
(15-18 months), the serum antibody titers to each *B. pertussis* antigen had declined
significantly but remained higher than pre-vaccination levels. One month following the
booster vaccination, antibody titers rose significantly to concentrations higher than post-
primary series levels. In addition, the proportion of subjects with seroconversion to *B.
pertussis* antigens increased from 81-92% post-primary series to 96-98% post-booster,
depending on the specific *B. pertussis* antigen (Table 2).

**Proliferative response.** Table 3 demonstrates T cell proliferative responses to *B.
pertussis* antigens. Prior to vaccination, none of the *B. pertussis* antigens induced a
positive proliferative response. Following the primary vaccination series, only PT and
PRN antigens induced positive proliferative responses with a median SI ≥ 3. The
frequency of post-primary positive proliferative response was highest for PT (67% of
subjects) and PRN (52%), and lowest for FHA (7%) and FIM (12%).
The proliferative response to PT decreased significantly by the pre-booster sampling point when compared to the post-primary series response. Following the booster vaccine, the proliferative response to PT antigen increased from a median SI of 1.7 to 3.3, and the proportion of subjects with positive PT-specific proliferative response increased from 37% to 54%. However, the post-booster proliferative response to FHA, PRN, and FIM antigens did not increase; the median SI was <3 for each of these antigens.

Overall, the proliferative response to FIM was very poor with a minority of subjects mounting a significant proliferative response post-primary series and none of the evaluable subjects mounting a positive proliferative response at the pre- or post-booster time-point. Of note, at the post-booster sampling point, there were fewer evaluable samples for the FIM antigen compared with other antigens (N = 18 for FIM compared to N = 21-37 for other antigens).

**Cytokine profile.** Cytokine secretion by antigen-stimulated PBMCs post-booster is summarized in Figure 2. After comparing *B. pertussis* antigen-induced cytokine production with cytokine levels without antigen stimulation, a significant increase in IFN-γ secretion in response to PT and FIM was noted (P=0.008 and 0.016, respectively). There was also a significant increase in IL-2 production in response to PT, FHA, and PRN antigens (P =0.001, <0.001, and =0.01, respectively). There was no statistically significant increase in IL-4 secretion in response to any studied antigens. We were unable to perform statistical analysis of IL-5 production because too few subjects’ PBMCs secreted detectable amounts of IL-5 in both unstimulated conditions and in
response to antigen stimulation. Subjects did produce IL-5 in response to mitogen stimulation, indicating that the assay conditions for cytokine measurement were satisfactory. There was significant increase in IL-10 production in response to PT and FHA antigens (P=0.01 and 0.018, respectively). TNF-α production did not increase significantly from baseline in response to any of the pertussis antigens.

Discussion

The majority of our study subjects demonstrated significant increases in antibody responses to all four *B. pertussis* antigens following the primary DTaP vaccination series. Antibody titers declined prior to the fourth dose (booster), but then increased significantly after the fourth dose, achieving higher antibody titers than after the primary vaccine series. The rapid decline in antibody titers prior to the booster dose has been illustrated in many studies (13, 22, 33) and supports the importance of a pertussis vaccine booster dose in the second year of life.

Although there is conflicting evidence regarding which *B. pertussis* antigens are considered most important for protection against disease (6, 34, 35), there is evidence that optimal anti-FIM antibody concentrations reduce short-term risk of pertussis in young children (36, 37). While PT, a key protective *B. pertussis* antigen, is a component of all current aP vaccines, FIM antigen is not present in all aP vaccines used globally (1, 9, 38, 39). Given recent evidence that PRN-deficient strains of *B. pertussis* are now circulating widely in the United States (40), and since our study revealed that FIM-containing aP vaccine was effective at inducing anti-FIM humoral response, the
inclusion of immunogenic FIM in vaccine preparations may be important for enhanced protection. Further studies examining anti-FIM antibody response are needed.

In our cohort, when comparing post-primary to pre-primary vaccination series samples, the proliferative response to PT and PRN antigens was positive in the majority of subjects, while only a minority of subjects mounted an adequate proliferative response to FHA and FIM. In contrast, Zepp et al. investigated proliferative responses one month after a primary series of a 3-component (PT, FHA, PRN) DTaP vaccine given at 3, 4, and 5 months, and reported a strong T cell proliferative response for all 3 pertussis antigens PT, FHA, and PRN (22). Unlike two previous studies (13, 22) reporting stable or even increased T cell proliferative responses measured at 12 to 14 months of age following a primary vaccination series with 3-component aP (13, 22), the children in our cohort revealed a decrease in proliferative response to PT and PRN prior to the booster series. Unexpectedly, following the booster vaccination at 15-18 months in our cohort, only a PT-specific response remained significant (median SI ≥ 3), while poor proliferative responses to the other B. pertussis antigens were observed. The differences in T cell proliferative response to various antigens observed between studies may be explained by varying antigen concentrations within the aP vaccines and slightly differing vaccination and sampling protocols.

Our analysis of the pattern of cytokine secretion in young infants is unique in that we investigated cytokine responses after the fourth dose of DTaP (post-booster, age 16-19 months), while other studies measured cytokine responses at various other time points. While interpreting cytokine secretion profiles, it is important to note that the
cytokine response to purified antigens may not exactly reflect the response to whole bacteria in *B. pertussis*-infected patients. Our study results suggest preferential induction of Th1 cytokines, as evidenced by a significant increase in IFN-γ production in response to PT and FIM antigens, and significant increase in IL-2 production in response to PT, FHA, and PRN antigens. The lack of significant increase in IL-4 secretion with any of the *B. pertussis* antigens and lack of IL-5 production in unstimulated and *B. pertussis* antigen-stimulated conditions suggest that our subjects lacked a significant Th2 response. This Th1 cytokine pattern is similar to that seen with wP and natural infection, and has been shown in humans and mice to be critical for clearance of pertussis infection (17, 19, 41). Studies in older children between 4 and 6 years of age (who had received 3-component primary aP vaccination) reported higher levels of Th1 cytokines IFN-γ and IL-2 compared to Th2 cytokines (11, 29). These authors suggested that given the relatively high exposure to *B. pertussis* in this Italian cohort, subclinical pertussis infection over time may have affected the immune response in these subjects. Other investigators (Zepp et al.) who noted a Th1-predominant cytokine profile in response to DTaP vaccine in infants used IL-10 as the sole marker for Th2 profile (21, 22). However, while IL-10 was previously considered a Th2 cytokine (particularly in mice), it is now known that in humans, IL-10 is not secreted by all Th2 cells and is produced by various cell types including Th1, Th2, regulatory T cells, and innate immune cells (26, 30). Since IL-10 is not an exclusive Th2 cytokine, conclusions about Th2 predominance cannot be made based on the lack of significant IL-10 production in the studies by Zepp et al. (21, 22) or the presence of significant IL-10 in response to PT and FHA antigens observed in our cohort.
More commonly, a Th2 or mixed Th1/Th2 cytokine profile has been reported with aP vaccination (16, 18, 20, 42) at various time points, including: 2 months after primary 2-component (PT and FHA) aP vaccination (16), 1 month following primary 3-component (PT, FHA, PRN) aP vaccine (42), and 2-4 years after primary 5-component (PT, FHA, PRN, FIM 2/3) aP vaccination (20). Studies also show that a DTaP booster administered between 4 and 6 years of age in children previously primed with DTaP induced a Th2 or mixed Th1/Th2 cytokine profile (20, 43, 44). A potential explanation for the difference in cytokine profile observed in our study population compared with other studies may be that cellular immunity during infancy may vary with age. Rowe et al. (45) analyzed tetanus-specific and polyclonal cytokine responses in infants from age 2-18 months. They found that Th2 cytokine response peaks at 12 months, then declines. Meanwhile, IFN-γ production (Th1) initially develops rapidly, declines around 6 months, remains low through age 12 months, and then resurges between 12-18 months. Since we measured cytokine responses at the post-booster period (16-19 months), it is possible that the cytokine profile observed in our subjects reflects the normal age-related variability of cellular immunity in infants. Furthermore, the significant levels of spontaneous IFN-γ secretion in this population may indicate an intrinsic ability of PBMCs to secrete IFN-γ at this stage.

Our study has a number of limitations. We only analyzed cytokine profiles following the booster vaccine, and we do not have pre-booster sample analysis to serve as a control. It would be important to measure cytokine secretion pre-booster in order to discriminate between responses specifically due to the vaccine booster (i.e. adaptive
immune response related to memory immune cells) versus a non-specific immune response. Therefore, our data do not rule out a non-specific immune response (perhaps age-related) that is not due to the vaccine itself. Further study is needed, measuring cytokine production both pre- and post-booster. In addition, the cytokine profile observed in our study may have been affected by antigens within vaccines co-administered with DTaP (e.g. IPV and Hib). As the AAP recommends that DTaP, IPV and Hib vaccinations be given at approximately the same time point, it may be impractical to administer only the DTaP vaccination without the other components of the Pentacel vaccine. Studies of non-vaccinated control subjects would not have been ethical since DTaP vaccines are recommended for all children. The interpretation of data for T cell proliferative response and cytokine production is limited by the fact that many samples were not evaluable due to the limited quantity of PBMCs recovered from some of the subjects, and priority for analysis was given first to PT, followed by FIM, PRN, and FHA antigens. It was particularly difficult to interpret cell-mediated and cytokine response to FIM because there were significantly fewer evaluable samples for FIM antigen. Although we did not specifically test for pertussis infection in this cohort, it is unlikely that the Th1 cytokine profile was due to subclinical pertussis infection during the study. From the post-primary to pre-booster sampling points, only four subjects had increase in antibody titer to FHA only, one had slightly increased titer to PT, and one had increased titers to all four antigens. While PT is a B. pertussis-specific antigen, FHA antigen is also found in Bordetella parapertussis and nonencapsulated Haemophilus influenzae strains (46-48). Therefore, while it is possible that two subjects may have
experienced subclinical pertussis during the study period, this is unlikely to fully explain our findings.

Our study has several strengths. Although it is often difficult to obtain sufficient blood samples for studies in infants, we were able to collect blood from a substantial number of children, including those younger than 6 months. Our study investigated immune response to the 5-component aP vaccine and examined immune response to four pertussis antigens, including FIM, which is often excluded in other studies. We measured several different Th1 and Th2 cytokines, thus allowing more complete examination of the pattern of cytokine secretion. We also examined Th1 and Th2 cytokines at a unique time point (1 month after the booster vaccination administered at 15-18 months), thus providing insight into infants’ immune response at an important stage in the pertussis vaccine schedule, as children do not receive their next aP vaccination until 4-6 years of age.

While it has been suggested that cell-mediated immune response may be a more reliable correlate of protection from pertussis infection than humoral response (22), the generally weaker T cell proliferative response to booster vaccination in our subjects supports the notion that the relative importance of each arm of the adaptive immune response may depend partly on the specific pertussis antigen against which the response is directed (49). It is often postulated that the failure of aP vaccine to induce a strong Th1 response is one explanation for the increasing incidence of pertussis infection (1). The Th1-consistent cytokine profile following aP booster vaccination in our
subjects supports the importance of a fourth vaccine dose at this age. This study suggests that the immune response induced by aP likely depends on several factors including the age of recipients, vaccination schedule, the balance of antigens within vaccines, and the individual host’s propensity for Th1 versus Th2 response. Recent animal studies indicate that another CD4+ T helper cell subset, Th17 cells, may also be important for controlling *B. pertussis* infection (2, 50). Larger studies are needed that investigate, among children primed with aP, a broad spectrum of aP-induced cytokines, including IL-17, at various time points including both pre- and post-booster. In addition, further studies are needed to determine the roles of various T cell subsets (Th1, Th2, and Th17) in protecting against human pertussis infection, as well as which antigens in the pertussis vaccine are most effective at eliciting protective immune response against pertussis.

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National Center for Advancing Translational Sciences or the National Institutes of Health.
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Table 1. Overview of study schedule and procedures

<table>
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<th>Estimated age</th>
<th>2 months (43-84 days)</th>
<th>4 months (90-152 days)</th>
<th>6 months (182-208 days)</th>
<th>7 months (209-237 days)</th>
<th>12 months (365-414 days)</th>
<th>15-18 months (439-537 days)</th>
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* Standard vaccines include Prevnar™, Hep B, and Varivax®.
The first dose of hepatitis B vaccine was given between birth and 1 month of age. Influenza vaccine, if indicated, was given to subjects as recommended by the American Academy of Pediatrics after six months of age (5). Hep B vaccine (Recombivax HB®): Merck & Co., Inc.; Prevnar™: Lederle laboratories, Pearl River, NY; M-M-R® II: Merck & Co., Inc., West Point, PA; Varivax®: Merck & Co., Inc., West Point, PA.
Table 2. Serum antibody response to *B. pertussis* antigens.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PT</th>
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<th>PRN</th>
<th>FIM</th>
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<tr>
<td></td>
<td>GMT (C.I.)</td>
<td>% with SC</td>
<td>GMT (C.I.)</td>
<td>% with SC</td>
</tr>
<tr>
<td>Pre-primary</td>
<td>2.3 (2.2-2.5)</td>
<td>3.9 (3.5-4.5)</td>
<td>2.8 (2.1-3.8)</td>
<td>8.9 (7.6-10.3)</td>
</tr>
<tr>
<td>(N=48)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-primary</td>
<td>25.2 (20.6-30.5)</td>
<td>49.3 (40.5-59.8)</td>
<td>39.7 (30-51.8)</td>
<td>157.0 (123.2-201.9)</td>
</tr>
<tr>
<td>(N=49)</td>
<td>92</td>
<td>92</td>
<td>81</td>
<td>85</td>
</tr>
<tr>
<td>Pre-booster</td>
<td>5.3 (4.3-8.8)</td>
<td>11.9 (9.4-15.5)</td>
<td>8.2 (6.3-11.2)</td>
<td>28.1 (22.0-37.4)</td>
</tr>
<tr>
<td>(N=47)</td>
<td>33</td>
<td>42</td>
<td>44</td>
<td>33</td>
</tr>
<tr>
<td>Post-booster</td>
<td>58.0 (46.3-72.3)</td>
<td>97.6 (78.9-124.0)</td>
<td>136.3 (96.6-185.5)</td>
<td>427.7 (313.8-576.9)</td>
</tr>
<tr>
<td>(N=48)</td>
<td>98</td>
<td>96</td>
<td>96</td>
<td>98</td>
</tr>
</tbody>
</table>

PT: pertussis toxin, FHA: filamentous hemagglutinin, PRN: pertactin, FIM: fimbriae types 2 and 3. Concentration of antibody specific to *B. pertussis* antigens (PT, FHA, PRN, FIM) is reported as geometric mean titer (GMT), with 95% bootstrap confidence intervals (C.I.). "% with SC represents the percentage of evaluated subjects with seroconversion (SC).
Table 3. T-cell Proliferative response to *B. pertussis* antigens

The magnitude of T cell proliferative responses was compared between the pre- and post-primary series, and between the post-primary and pre-booster time points, by Wilcoxon signed rank test. *P* value ≤ 0.05 is considered statistically significant.

**% + CMI represents the percentage of subjects with positive cell-mediated immune response (i.e., SI ≥ 3).**

<table>
<thead>
<tr>
<th>Sample</th>
<th>PT</th>
<th>FHA</th>
<th>PRN</th>
<th>FIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-primary</td>
<td>N</td>
<td>SI: L/M/U*</td>
<td>% + CMI***</td>
<td>N</td>
</tr>
<tr>
<td>34</td>
<td>0.9, 1.0, 1.2</td>
<td>0%</td>
<td>28</td>
<td>0.1, 0.2, 0.3</td>
</tr>
<tr>
<td>Post-primary</td>
<td>33</td>
<td>2.5, 3.9, 5.8</td>
<td>&lt;0.001</td>
<td>67%</td>
</tr>
<tr>
<td>Pre-booster</td>
<td>43</td>
<td>1.2, 1.7, 3.2</td>
<td>0.032</td>
<td>37%</td>
</tr>
<tr>
<td>Post-booster</td>
<td>37</td>
<td>1.3, 3.3, 5.1</td>
<td>54%</td>
<td>29</td>
</tr>
</tbody>
</table>

*SI is presented as median with interquartile range. L/M/U indicates lower quartile, median, upper quartile.
Figure legends

**Figure 1.** Trend for antibody response to each *B. pertussis* antigen during vaccination series

Antibody titers are reported as geometric mean titer (GMT) with 95% confidence intervals.

**Figure 2.** Cytokine secretion by antigen-stimulated PBMCs, measured 1 month following aP booster

Cytokine (IFN-γ, IL-2, IL-10, IL-4) production in response to pertussis antigens (PT, FHA, PRN, FIM) and in unstimulated conditions ("unstim") was compared by Wilcoxon signed rank test. Cytokine levels are plotted as a box and whisker plot. The bottom and top of the box represent the first and third quartiles respectively, and the horizontal band inside the box represents the median. The ends of the whiskers represent the minimum and maximum values excluding outliers. A two-tailed *P* value ≤0.05 was considered to represent a significant increase in cytokine production in response to the tested antigen.
Figure 1. Trend for antibody response to each B. pertussis antigen during vaccination series.

Antibody titers are reported as geometric mean titer (GMT) with 95% confidence intervals.
Figure 2. Cytokine secretion by antigen-stimulated PBMCs, measured 1 month following aP booster.

Cytokine (IFN-γ, IL-2, IL-10, IL-4) production in response to pertussis antigens (PT, FHA, PRN, FIM) and in unstimulated conditions (‘unstim’) was compared by Wilcoxon signed rank test. Cytokine levels are plotted as a box and whisker plot. The bottom and top of the box represent the first and third quartiles respectively, and the horizontal band inside the box represents the median. The ends of the whiskers represent the minimum and maximum values excluding outliers. A two-tailed P value <0.05 was considered to represent a significant increase in cytokine production in response to the tested antigen.