Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model

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Pertussis is a highly contagious respiratory illness caused by the bacterial pathogen *Bordetella pertussis*. Pertussis rates in the United States have been rising and reached a 50-y high of 42,000 cases in 2012. Although pertussis resurgence is not completely understood, we hypothesize that current acellular pertussis (aP) vaccines fail to prevent colonization and transmission. To test our hypothesis, infant baboons were vaccinated at 2, 4, and 6 mo of age with aP or whole-cell pertussis (wP) vaccines and challenged with *B. pertussis* at 7 mo. Infection was followed by quantifying colonization in nasopharyngeal washes and monitoring leukocytosis and symptoms. Baboons vaccinated with aP were protected from severe pertussis-associated symptoms but not from colonization, did not clear the infection faster than naïve animals, and readily transmitted *B. pertussis* to unvaccinated contacts. Vaccination with wP induced a more rapid clearance compared with naïve and aP-vaccinated animals. By comparison, previously infected animals were not colonized upon secondary infection. Although all vaccinated and previously infected animals had robust serum antibody responses, we found key differences in T-cell immunity. Previously infected animals and wP-vaccinated animals possess strong *B. pertussis*-specific T helper 17 (Th17) memory and Th1 memory, whereas aP vaccination induced a Th1/Th2 response instead. The observation that aP, which induces an immune response mismatched to that induced by natural infection, fails to prevent colonization or transmission provides a plausible explanation for the resurgence of pertussis and suggests that optimal control of pertussis will require the development of improved vaccines.

whooping cough | T-cell memory | animal models | adaptive immunity | IL-17

Pertussis is a highly contagious, acute respiratory illness caused by the bacterial pathogen *Bordetella pertussis* (1, 2). Infection results in a wide spectrum of clinical manifestations ranging from mild respiratory symptoms to a severe cough illness accompanied by marked leukocytosis and the hallmark inspiratory whoop and posttussive emesis (3). Because acellular pertussis vaccines replaced whole-cell vaccines in the 1990s, pertussis has reemerged at a startling rate in the United States despite nationwide vaccine coverage in excess of 95% (4). With a 50-y high of 42,000 reported cases in the United States in 2012, pertussis is the most common of the vaccine-preventable diseases (5). This resurgence is mirrored throughout the industrial world despite similar high rates of vaccination (6–9). Two common hypotheses for the resurgence have been proposed: *i*) current acellular pertussis vaccines (aP) vaccines are less effective than the whole-cell pertussis (wP) vaccines they replaced and *ii*) aP-induced immunity wanes more quickly than anticipated (10–13). However, pertussis resurgence is not completely understood (14, 15).

Hampering our ability to counteract this resurgence is the fact that pertussis pathogenesis and immunity to natural infection have not been well studied in humans because typical pertussis is sporadic given high rates of vaccination in developed countries. Human challenge studies have been proposed but never conducted due to a variety of logistical and ethical problems including the potential for severe disease, the lack of an effective therapeutic for established disease, and the highly contagious nature of pertussis. Although a variety of small-animal models have been used to study pertussis, none of them adequately reproduce the human disease (16). To address this gap, we recently developed a nonhuman primate model of pertussis using baboons (*Papio anubis*) and found the disease is very similar to severe clinical pertussis. Upon challenge, baboons experience 2 wk of heavy respiratory colonization and leukocytosis peaking between 30,000–80,000 cells/mL, similar to the range in pertussis-infected infants (1, 17). In addition, baboons experience a paroxysmal cough illness characterized by repeated fits of 5–10 coughs. The coughing fits last on average >2 wk in the baboon, although this is less than some severely infected children, where the cough can last up to 12 wk (1, 17). We also characterized airborne transmission of *B. pertussis* from infected to naïve animals, which is the route of transmission postulated to occur between humans (18). Because this is the only model of pertussis to reproduce the cough illness and transmission of the human disease, we believe it provides the unique opportunity to test our hypothesis that aP vaccines fail to prevent *B. pertussis* colonization, thus enabling transmission among vaccinated individuals.

Using this model we have confirmed that, as in humans, aP vaccines provide excellent protection against severe disease in baboons. However, aP vaccines do not prevent colonization following direct challenge or infection by transmission. In addition, aP-vaccinated animals are capable of transmitting disease to naïve contacts. By comparison, wP-vaccinated animals cleared infection significantly more quickly than aP-vaccinated or naïve

Significance

Pertussis has reemerged as an important public health concern since current acellular pertussis vaccines (aP) replaced older whole-cell vaccines (wP). In this study, we show nonhuman primates vaccinated with aP were protected from severe symptoms but not infection and readily transmitted *Bordetella pertussis* to contacts. Vaccination with wP and previous infection induced a more rapid clearance compared with naïve and aP-vaccinated animals. While all groups possessed robust antibody responses, key differences in T-cell memory suggest that aP vaccination induces a suboptimal immune response that is unable to prevent infection. These data provide a plausible explanation for pertussis resurgence and suggest that attaining herd immunity will require the development of improved vaccination strategies that prevent *B. pertussis* colonization and transmission.

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animals. We also found that aP vaccination induces T helper 2 (Th2) and T helper 1 (Th1) immune memory responses, whereas infection and—to a lesser extent—wP vaccination induce Th17 and Th1 memory. Our results suggest that in addition to the potential contribution of reduced efficacy and waning immunity of aP, the inability of aP to prevent colonization and transmission provides a plausible explanation for pertussis resurgence.

Results

Acellular Pertussis Vaccines Protect Against Disease but Fail to Prevent Infection. Several observational studies recently concluded that children primed with aP vaccine are at greater risk for pertussis diagnosis compared with wP-primed children (19–22). Although these data suggest aP vaccine is less effective than wP vaccine at preventing colonization, the rate of undiagnosed *B. pertussis* carriage in vaccinated individuals is unknown. To assess the ability of each vaccine to prevent colonization and clinical pertussis symptoms, baboons were vaccinated according to the US schedule at 2, 4, and 6 mo of age with human doses of combination diphtheria, tetanus, and pertussis vaccines containing aP or inactivated wP (Table 1 provides a list of the components of each vaccine). At 7 mo of age, vaccinated, naïve, and previously infected (convalescent) animals were challenged with D420, a *B. pertussis* clinical isolate that causes severe infection in humans and baboons (17). Naïve animals were heavily colonized with peak levels between 10^7–10^8 cfu/mL in nasopharyngeal washes (Fig. 1*A*). After 2 wk, colonization gradually decreased, and the infection cleared after 30 d. Consistent with our previous finding, none of the convalescent animals were colonized (17). Compared with naïve animals, aP-vaccinated animals had slightly reduced colonization for the first 10 d but remained consistently colonized before clearing after 35 d. In wP-vaccinated animals the initial colonization was similar to aP-vaccinated animals but the infection cleared after 18 d, significantly faster than naïve and aP-vaccinated animals (Fig. 1*B*).

To assess the efficacy of the vaccines in preventing the symptoms of severe pertussis, peripheral blood was drawn serially, and complete blood counts were performed to monitor leukocytosis, a significant marker of morbidity in pertussis-infected infants (23). Compared with preinfection levels, naïve animals had a significant increase in circulating white blood cells at each time point, peaking at over 40,000 cells per µL, an eightfold increase over preinfection levels (Fig. 1*C*). In contrast to the colonization data, aP vaccination, wP vaccination, and convalescence all prevented leukocytosis (Fig. 1*C*). In addition, wP-vaccinated, aP-vaccinated, and convalescent animals did not cough and showed no reduction of activity, loss of appetite, or other outward signs of disease.

Acellular Vaccines Fail to Prevent Infection Following Natural Transmission. To assess the ability of vaccination to prevent pertussis infection by transmission, two aP-vaccinated animals and one unvaccinated animal were cohoused with a directly challenged, unvaccinated animal. Similar to our previous findings (18), all animals became colonized 7–10 d after cohousing with the infected animal (Fig. 2). The peak levels and kinetics of colonization were indistinguishable between the naïve and aP-vaccinated animals.

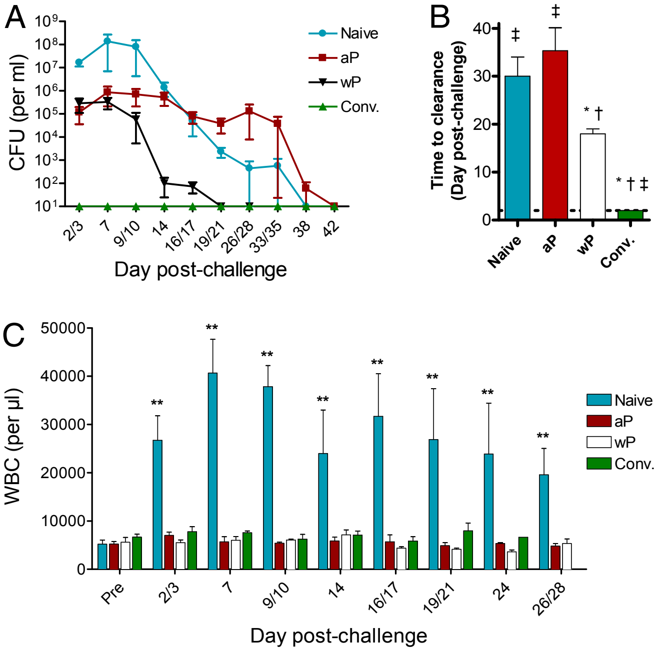

Fig. 1. The effect of vaccination or convalescence on colonization and leukocytosis. Naïve animals, aP-vaccinated animals, wP-vaccinated animals, and previously infected [convalescent conv.)] animals were directly challenged with *B. pertussis* ($n = 3$–4 per group). (*A*) Colonization was monitored by quantifying *B. pertussis* cfu per mL in biweekly nasopharyngeal washes with a limit of detection of 10 cfu per mL. For each animal the time to clearance is defined as the first day that no *B. pertussis* cfu were recovered from nasopharyngeal washes. (*B*) The mean time to clearance is shown for each group ($n = 3$ per group). Because no *B. pertussis* organisms were recovered from the conv. animals, the mean time to clearance was defined as the first day of sampling (day 2, indicated by the dashed line). *$P < 0.05$ vs. Naïve, †$P < 0.05$ vs. aP, ‡$P < 0.05$ vs. wP. (*C*) The mean circulating white blood cell counts before and after challenge are shown for each group of animals ($n = 3$–4 per group). **$P < 0.01$ vs. preinfection from same group.

Acellular-Vaccinated Animals Are Capable of Transmitting *B. pertussis* to Naïve Contacts. Because aP fails to prevent colonization we hypothesized that aP-vaccinated animals can transmit *B. pertussis* infection to contacts. To test this hypothesis, two aP-vaccinated animals were challenged with *B. pertussis* and placed in separate cages. After 24 h, a naïve animal was added to each cage, and all animals were followed for colonization. Both of the naïve animals were infected by transmission from their aP-vaccinated cage mates (Fig. 3).

Vaccination and Previous Infection Induce Robust Antibody Responses. Sera collected before vaccination or primary infection and again at 1 wk before challenge were analyzed for IgG antibodies against heat-killed *B. pertussis* and the vaccine antigens

Table 1. Components of aP and wP vaccines used in this study

Vaccine component	Daptacel	Infanrix	Triple antigen
Diphtheria toxoid	15 Lf	25 Lf	20–30 Lf
Tetanus toxoid	5 Lf	10 Lf	5–25 Lf
Whole-cell *Bordetella pertussis*	—	—	≥4 IU
Inactivated pertussis toxin	10 µg	25 µg	—
Filamentous hemagglutinin	5 µg	25 µg	—
Pertactin	3 µg	8 µg	—
Fimbriae types 2 and 3	5 µg	—	—
Aluminum (from aluminum phosphate)	0.33 mg	≤0.625 mg	≤1.25 mg

IU, international units; Lf, limit of flocculation units.

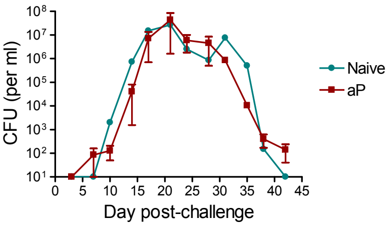

Fig. 2. aP does not protect against colonization following natural transmission. A naïve animal was directly challenged. After 24 h, a naïve animal and two aP-vaccinated animals were placed in the same cage as the directly challenged animal and followed for colonization as in Fig. 1.

pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae types 2 and 3 (FIM). We show that wP, aP, and natural infection induce high-antibody titers to all antigens, and the aP group generally possessed equivalent or greater prechallenge titers, suggesting that the differences in colonization between the groups do not correlate with levels of circulating antipertussis antibodies (Fig. 4). Following challenge, the titers for vaccinated animals were essentially unchanged, whereas boosting was observed for some antigens in convalescent animals (Fig. S1).

T-Cell Memory Response Elicited by Acellular Pertussis Vaccination Is Mismatched Compared with Natural Infection. Although a large number of clinical studies have characterized the antibody response to pertussis infection and vaccination, key deficiencies remain in our understanding of pertussis-induced helper T-cell immune responses in humans and primates. Importantly, no clinical studies have investigated whether the primary series of pertussis vaccines induce Th17 memory, a recently identified T cell that specializes in controlling extracellular bacterial infections at mucosal surfaces through stimulating neutrophil recruitment (24). To assess *B. pertussis*-specific T-cell memory responses in naïve, aP-vaccinated, wP-vaccinated, and convalescent animals, peripheral blood mononucleated cells (PBMCs) were collected 1 wk before infection. Total PBMC were incubated either with medium alone or with heat-killed *B. pertussis* as an ex vivo simulation of the memory responses recalled during the ensuing challenge. Following an overnight incubation, nonadherent PBMC, including T cells, were collected and separated using magnetic beads into the following fractions: CD4−, CD4+, CD95–CD4+, or left unseparated (total nonadherent cells). Memory helper T cells in primates are characterized by surface expression of CD4 and CD95 (25, 26). After further culture of all fractions, the supernatants were analyzed for secretion of IL-17, IFN-γ, and IL-5; cytokines that are characteristic of Th17, Th1, and Th2 cells, respectively. Very low background cytokine secretion was observed from nonstimulated cells isolated from naïve, vaccinated, or convalescent animals or from stimulated cells from naïve animals (Figs. S2 and S3). When stimulated with heat-killed *B. pertussis*, both total nonadherent cells and CD4+ cells from convalescent animals secreted high levels of IL-17, some IFN-γ, and no IL-5. When the CD95+ memory cells were depleted, the CD95–CD4+ cells did not secrete IL-17 or IFN-γ, consistent with induction of *B. pertussis*-specific Th17 and Th1 memory cells (Fig. 5). Stimulated total nonadherent cells and CD4+ cells from aP-vaccinated animals secreted significant IFN-γ, but the response was weaker than convalescent cells ($P = 0.01$), and there was no significant increase in IL-17 secretion. However, there was a significant IL-5 response, consistent with skewing toward Th2 and Th1 memory (Fig. 5). Total nonadherent cells and CD4+ cells from wP-vaccinated animals secreted similar IFN-γ compared with aP cells, but no IL-5. IL-17 secretion was between levels for naïve and convalescent cells, suggesting that T-cell memory induced by wP vaccination is similar to natural infection, but the Th17 and Th1 memory responses were weaker.

Discussion

The introduction of whole-cell vaccines consisting of inactivated *Bordetella pertussis* organisms in the United States in the 1940s caused a precipitous decrease in pertussis incidence (27). However, over the past 30 y, pertussis has resurged in the United States. The resurgence began during the wP vaccine era, but the pace has quickened since aP vaccines were recommended for all primary and booster doses (11). This correlation has led many to hypothesize that aP vaccines are less effective on a population scale than the wP vaccines they replaced (10, 12, 13). Consistent with this notion, several recent observational studies concluded that children primed with aP vaccine had a twofold to fivefold greater risk of pertussis diagnosis compared with wP-primed children (19–22). Our results in nonhuman primates add to these findings by showing that animals vaccinated with wP cleared infection by a direct challenge twice as fast as animals vaccinated with aP. However, neither vaccine was able to prevent colonization as well as immunity from a previous infection.

Another hypothesis as to why pertussis is reemerging is that the duration of immunity in aP-vaccinated children is shorter than anticipated. Although some first-generation acellular vaccines had poor immunity and efficacy, double-blinded clinical trials and field-efficacy studies for the US-licensed acellular vaccines estimated the short-term efficacy to be excellent: ∼85% after three doses and 98% after five doses (28–30). However, recent cohort and case-control studies concluded that 5 y following the fifth aP dose, children are fourfold to 15-fold more likely to acquire pertussis compared with within the first year, consistent with waning aP immunity (30–33).

We hypothesized an additional explanation for pertussis resurgence is that aP-vaccinated individuals can act as asymptomatic or mildly symptomatic carriers and contribute significantly to transmission in the population. Observational studies suggest that asymptomatic pertussis can occur in vaccinated children and adults based on PCR or serological data (34, 35). However, during the aP vaccine trials, participants were not screened for *B. pertussis* infection unless they presented with pertussis-like symptoms and at least 7–21 d cough (12). Therefore, no experimental data exist on whether vaccination prevents *B. pertussis* colonization or transmission in humans. In the present study we show that aP-vaccinated primates were heavily infected following direct challenge, and the time to clearance was not different compared with naïve animals. Similarly, there was no difference in the kinetics or peak level of colonization between aP-vaccinated and naïve animals that were infected by natural transmission. Importantly, we also show in two experiments that aP-vaccinated animals transmitted *B. pertussis* to naïve cage mates. Together these data form the key finding of this study: aP vaccines do not prevent infection or

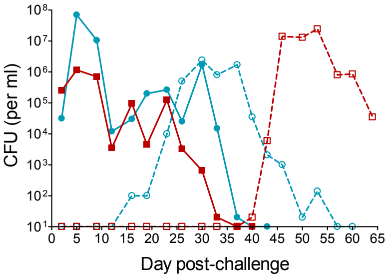

Fig. 3. Infected aP vaccinees can transmit pertussis to naïve contacts. Two animals vaccinated with aP were housed in separate cages, and each was directly challenged. Twenty four hours after challenge, an unchallenged naïve animal was placed in each cage. All animals were followed for colonization as in Fig. 1. One cage pairing is shown with turquoise lines with circles, and the other is shown with maroon lines with squares. Solid lines with closed symbols indicate the aP-vaccinated, directly challenged animals, and open symbols with dashed lines are used for the unchallenged, naïve contacts.

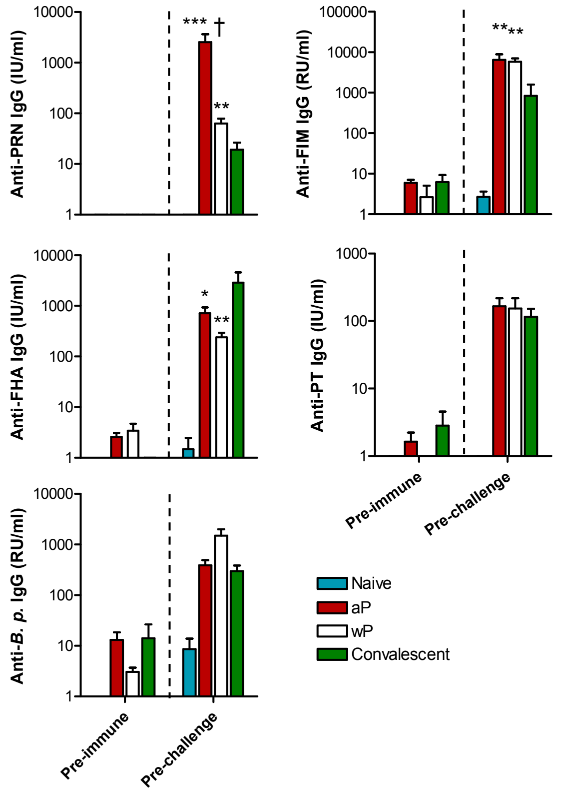

Fig. 4. Vaccination and previous infection induce robust serum antibody responses. Antibody responses to the four vaccine antigens—PRN, FIM, FHA, and PT—and to heat-killed *B. pertussis* (*B. p.*) were measured by ELISA. Preimmune sera were collected from vaccinated animals before immunization and from conv. animals before initial infection (n = 3–4 per group). Because Infanrix does not contain FIM, four Daptacel-vaccinated animals were included in the anti-FIM ELISA. Prechallenge sera were collected from all animals 1 wk before challenge. International Units (IU) or relative units (RU) in each sample were determined by comparing the responses to the WHO international standard pertussis antiserum on each plate. ***$P <$ 0.001, **$P < 0.01$, *$P < 0.05$ vs. Convalescent. †$P < 0.001$ vs. wP.

transmission of *Bordetella pertussis* even 1 mo after completing the primary vaccination series.

We show that wP, aP, and natural infection all induce high-antibody titers. The prechallenge titers in aP-vaccinated animals were generally equivalent or higher than those observed in convalescent and wP-vaccinated animals, suggesting that aP is immunogenic in baboons and that the inability to prevent infection was not due to low-antibody titers. Compared with the large number of clinical studies that have characterized the antibody response to pertussis infection and vaccination, very few have investigated pertussis-induced helper T-cell immune responses in humans. Taken as a whole, these limited data suggest that aP vaccination induces Th2 or mixed Th2/Th1 responses, whereas wP vaccination and natural infection induce a Th1 response (13). However, none of these studies tested for Th17 memory, a recently identified T cell that specializes in controlling extracellular bacterial infections at mucosal surfaces (24). Our data show that natural infection induced robust Th17 and Th1 immunity. Animals vaccinated with wP, which cleared infection faster than naïve and aP-vaccinated animals, showed similar but weaker T-cell responses. wP vaccination is generally believed to induce strong Th1 responses, but what we observed here was relatively weak. This observation might be explained by heterogeneity in the manufacturing of different wP vaccines. Future studies will compare the immune response induced by wP vaccines produced by three different manufacturers. In comparison with natural infection and wP, aP-induced immunity was mismatched, showing a Th2 response with a weaker Th1 response and no significant Th17 response.

Together, the cytokine and T-cell immunological data observed in baboons are generally consistent with those observed in mice (13). We previously showed that pertussis infection in baboons induces a mucosal immune response characterized by production of IL-17 and a variety of chemokines and cytokines associated with IL-17 signaling, including IL-6 and IL-8. This primary immune response correlated with long-lived Th17 and Th1 memory responses that lasted >2 y (36). Mice infected with *B. pertussis* also express mucosal IL-17, IL-6, and IL-8 homologs and induce Th17 and Th1 memory (37–40). Mice vaccinated with wP also develop Th17 and Th1 memory that results in partial protective immunity, similar to what we observed in the baboon model (41, 42). A recent report by Ross et al. (42) concluded that an aP containing PT, FHA, and PRN induces Th1, Th2, and Th17 immune responses in C57BL/6 mice (42). However, a previous study from the same group found Th1 and Th2 but no

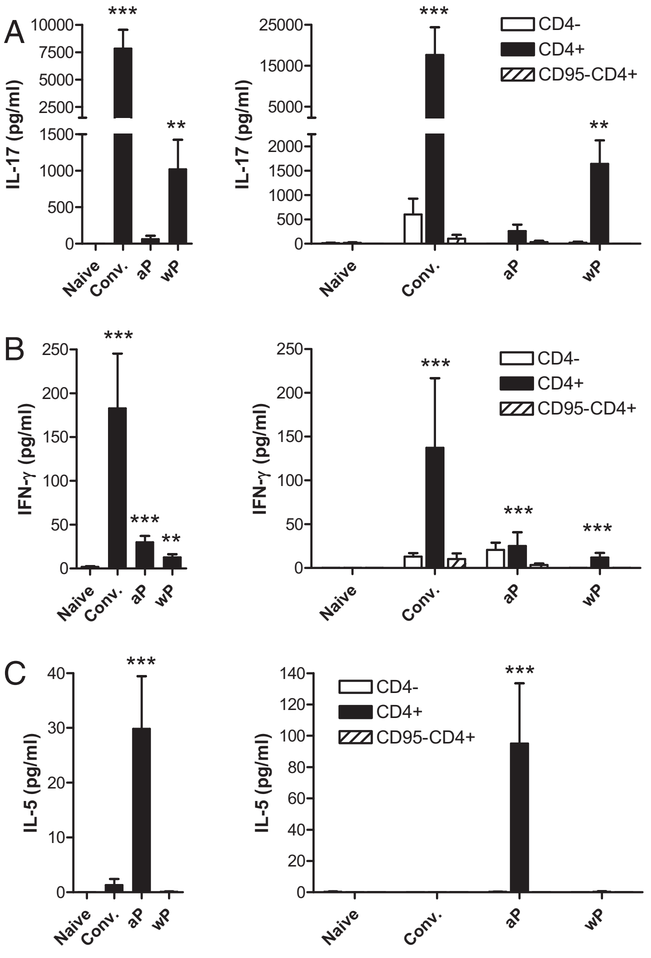

Fig. 5. Helper T-cell responses induced by vaccination and infection. PBMC collected from naïve, aP-vaccinated, wP-vaccinated, and conv. animals 1 wk before infection were incubated overnight with either medium alone or medium containing heat-killed *B. pertussis* (n = 3–4 per group). For each growth condition, nonadherent cells were collected and either left unseparated (total nonadherent cells) or separated using anti-CD4 and anti-CD95 magnetic particles. Total nonadherent, CD4−, CD4+, and CD95−CD4+ cells were then cultured under the same conditions as before (with medium alone or stimulated with heat-killed *B. pertussis*). After 36 h, supernatants were collected and analyzed for IL-17 (*A*), IFN-γ (*B*), and IL-5 (*C*). Cytokine secretion in response to *B. pertussis* stimulation is presented for total nonadherent cells (*Left*) and separated cells (*Right*). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ vs. same fraction from naïve animals.

significant Th17 responses in C3H/HeJ and C3H/HeN mouse strains vaccinated with an aP containing PT and FHA (41). Nevertheless, data from two clinical studies recently showed negligible Th17 recall responses (~10 pg/mL) in PBMC isolated from aP-vaccinated 4-y-old children before and after booster, suggesting aP does not induce Th17 memory in humans (43, 44).

Taken as a whole, the data presented in this study suggest that antibodies induced by aP vaccination are sufficient for preventing severe pertussis symptoms but do not mitigate colonization. Inhibition of leukocytosis likely occurs through antibody-mediated neutralization of PT, a toxin which interferes with leukocyte extravasation by blocking chemokine receptor signaling (1). The mechanism by which aP prevents coughing despite heavy bacterial colonization is not known but deserves further attention. On the other hand, induction of Th17/Th1 memory responses correlated with the ability to clear infection: convalescent and wP-vaccinated animals possessed strong Th17 responses and Th1 responses and cleared infection more quickly than aP-vaccinated animals which lacked Th17 responses but possessed Th1/ Th2 memory. Although we have not definitively shown that Th17 cells are required for *B. pertussis* clearance, this correlation is consistent with the role these cells play in fighting extracellular bacterial infections at mucosal surfaces by inducing neutrophil chemotaxis. The current studies were not designed to look at immune cell recruitment to the respiratory tract, but additional experiments are underway to determine the role of neutrophils in the immune response to pertussis infection and vaccination in baboons. We are also investigating other possible mechanisms that could prevent mucosal colonization; for example, a possible role for IgA and IgD which are secreted in primate lower and upper respiratory tracts, respectively (45, 46).

The baboon model offers many advantages, chiefly the ability to investigate pertussis pathogenesis, transmission, and host immune responses to infection and vaccination in a primate species that is >96% genetically similar to humans (47). However, there are also several limitations associated with this model. There are far fewer animals available for research compared with smaller-animal models. In addition, there is a paucity of immunological reagents that are validated for baboons compared with mice and humans. Although antibodies against cell surface markers are generally cross-reactive, anti-cytokine antibodies tend to be much more species-specific. For this reason we have so far been unable to assess T-cell responses using intracellular cytokine staining and flow cytometry. This led us to develop the cell separation assay as an alternative method for phenotyping the memory T-cell responses induced by pertussis infection and vaccination (36). One limitation of our assay is that during the CD4+ cell purification, antigen-presenting cells such as macrophages and dendritic cells are removed after an overnight incubation. This likely explains the low IFN-γ secretion observed in all groups because antigen-presenting cells increase IFN-γ secretion by antigen-specific CD4+ T cells through a positive feedback loop (48). In line with this hypothesis, our previous data showed that restimulated whole PBMC from convalescent animals secreted much higher levels of IFN-γ. In addition, restimulation assays using human PBMC or murine splenocytes after infection or vaccination also show higher levels of secreted IFN-γ (42, 49). Together these observations suggest that although our assay is valuable for phenotyping T-cell memory, it likely underrepresents the magnitude of Th1 memory responses. We used heat-killed *B. pertussis* as an antigen for our restimulation assays because we believe this is the most relevant method for ex vivo simulation of T-cell memory recalled during infection. However, it is possible that this assay underdetects immune responses that would be observed had we used purified vaccine antigens. Another disadvantage of primate models is that it is not feasible to directly link an immune response to protection. Although protection from pertussis has been shown to be mediated by IFN-γ and, to a lesser extent, IL-17 signaling using knockout mouse strains lacking specific gene products (13), the relative protection afforded by Th17 or Th1 responses in vaccinated or convalescent baboons or humans is not known.

Currently, a major focus of public health agencies is the prevention of pertussis infection in young infants who have not completed their primary aP series and have considerable morbidity and mortality to pertussis infection (1). One recommendation to reduce transmission of pertussis to infants is by "cocooning," or vaccinating people who have contact with infants (11). Our data show that aP-vaccinated animals are infected and transmit pertussis to naïve contacts. Consistent with these findings, seroepidemiological studies have concluded that *B. pertussis* circulation is still high in countries with excellent aP uptake (27, 50), and a cross-sectional study showed that postpartum aP vaccination of mothers did not reduce pertussis illness in young infants (51). These data suggest that cocooning is unlikely to be an effective strategy to reduce the burden of pertussis in infants. However, it is important to note that our data in combination with human data show that vaccination with aP provides excellent protection from severe pertussis (52). Therefore, any short-term plan for addressing the resurgence of pertussis should include continued efforts to enhance aP immunization. However, to protect the most vulnerable members of the population and achieve optimal herd immunity, it will be necessary to develop a vaccination strategy that effectively blocks pertussis infection and transmission.

Materials and Methods

Ethics Statement. All animal procedures were performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International in accordance with protocols approved by the Center for Biologics Evaluation and Research Animal Care and Use Committee and the principles outlined in the *Guide for the Care and Use of Laboratory Animals* by the Institute for Laboratory Animal Resources, National Research Council (53).

Bacterial Strains and Media. *B. pertussis* strain D420 was grown on Bordet–Gengou and Regan–Lowe plates prepared as described previously (17). Heat-killed *B. pertussis* was prepared by resuspending to an OD_{600} of 0.90 (5×10^9 cfu/mL) in PBS and heating at 65 °C for 30 min.

Vaccination, Infection, and Evaluation of Baboons. Baboons obtained from the Oklahoma Baboon Research Resource at the University of Oklahoma Health Sciences Center were inoculated with human doses of aP or wP administered intramuscularly at 2, 4, and 6 mo of age. For studies using aP, equal numbers of animals were vaccinated with Daptacel (Sanofi Pasteur Ltd.) and Infanrix (GlaxoSmithKline). For wP, animals were vaccinated with Triple Antigen (Serum Institute of India Ltd.), which meets the World Health Organization (WHO) recommendations for potency. Naïve animals were age-matched but not vaccinated. Previously infected animals were clear of *B. pertussis* infection for 1 to 2 mo before reinfection. Direct challenge and transmission studies were performed as described previously (17, 18). The inoculum for each direct challenge was between 10^9–10^{10} cfu as determined by measurement of optical density and confirmed by serial dilution and plating to determine the number of cfu per mL of inoculum. Baboons were evaluated twice weekly as described previously for enumeration of circulating white blood cells and serum separation (17). Nasopharyngeal washes were diluted and plated on Regan–Lowe plates to quantify bacterial cell counts.

Isolation of PBMC and Cell Separation. Baboons were anesthetized, and PBMC were isolated from peripheral blood as described previously (36) and cryopreserved in RPMI-1640 medium supplemented with 10% (vol/vol) DMSO and 12.5% (wt/vol) BSA using Mr. Frosty containers (Nalgene). After thawing, cells were washed twice and nonadherent cells were collected as described previously. For each growth condition, cells were incubated overnight with either medium alone or medium containing heat-killed *B. pertussis* (50 bacteria:1 PBMC). Nonadherent cells were collected, and 2×10^6 cells were left unseparated (total nonadherent cells). Using the method previously described, 4×10^6 cells were separated using anti-CD4 magnetic particles, and another 4×10^6 cells were depleted of CD95+ cells and then separated with anti-CD4 magnetic particles (36). The following fractions were collected: Total nonadherent, CD4−, CD4+, and CD95–CD4+. After incubation with or without heat-killed *B. pertussis*, cells were pelleted and supernatants were collected for IL-17A quantitation by ELISA (Aniara) and quantitation of IFN-γ and IL-5 using the Milliplex MAP nonhuman primate kit according to the manufacturer's instructions (Millipore). Data are presented as the cytokine concentration secreted by *B. pertussis*-stimulated cells minus the basal concentration secreted by cells incubated with medium alone.

Detection of Serum Antibodies to Pertussis Antigens. Nunc Maxisorp 96-well plates were coated overnight with 0.2 μg/mL PT, 0.5 μg/mL FHA, 2 μg/mL PRN, or 0.2 μg/mL FIM (List Biologicals) as described previously (17, 54). For whole-bacteria ELISA, plates were coated overnight at 37 °C with heat-killed *B. pertussis* prepared as described above. Serum IgG for each antigen was measured as described previously (17). Each plate contained a standard curve from the WHO international standard pertussis antiserum (National Institute for Biological Standards and Control) used to assign international units for PT, FHA, and PRN and relative units for FIM and heat-killed *B. pertussis* by comparison with the linear portion of the standard curve. Because Infanrix does not contain FIM, only Daptacel-vaccinated animals were included in the anti-FIM ELISA.

Statistics. All data are reported as mean ± SEM. Statistical analyses were performed by ANOVA with post hoc *t* test using JMP (version 9) software (SAS Institute, Inc.). Antibody and cytokine data were normalized by log transformation before analysis.

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