EXPERT REVIEWS

The baboon model of pertussis: effective use and lessons for pertussis vaccines

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The USA is experiencing a pertussis resurgence that resulted in a 60-year high of 48,000 cases in 2012. Our ability to counteract this resurgence is hampered by the fact that pertussis pathogenesis and immunity to pertussis infection are not well studied. Studies in humans are difficult due to the low frequency of pertussis in the population, the cyclical nature of incidence and the sporadic geographic distribution of cases. While existing animal models reproduce many aspects of pertussis, none of them adequately reproduces the full spectrum of disease. We describe the baboon model of pertussis. The baboon model is the first animal model that recapitulates the full spectrum of human pertussis including coughing and transmission. This model is being utilized to examine pertussis pathogenesis and host responses to infection and vaccination. It is likely the baboon model will provide an important tool in the development of improved pertussis vaccines.

KEYWORDS: baboons • pertussis • T-cell memory • Th17 • vaccines • whooping cough

History of pertussis disease & vaccination in the USA

Pertussis is a highly contagious, acute respiratory illness caused by the bacterial pathogen *Bordetella pertussis* [1,2]. Classically defined pertussis consists of three stages. The catarrhal stage consists of rhinorrhea, malaise and low-grade fever. Over a period of approximately 1–2 weeks, the disease progresses to the paroxysmal stage, characterized by severe coughing fits. These coughing paroxysms may be accompanied by the classic inspiratory whoop and post-tussive emesis. Clinical symptoms include high leukocytosis, hypoglycemia and reduced pulmonary capacity. Symptoms slowly wane and gradually resolve in the convalescent phase. Complications associated with *B. pertussis* infection include conjunctival hemorrhage, inguinal hernia, pneumonia, respiratory distress, seizures, encephalopathy, apnea and death. The highest incidence of disease is in infants too young to have completed primary vaccinations and the vast majority of severe pertussis cases requiring hospitalization and fatal cases occur in infants less than 3 months of age [3,4].

Before the advent of vaccines, pertussis was a major burden in the USA, with reported cases ranging from 100,000 to 265,000 per year for the first half of the 20th century [5]. In the 1940s, the introduction of whole-cell vaccines consisting of inactivated *B. pertussis* organisms led to a precipitous decrease in pertussis incidence [6]. However, concerns over side effects from combination diphtheria, tetanus and whole-cell pertussis (wP) vaccines prompted the development of diphtheria, tetanus and acellular pertussis (aP) vaccines consisting of purified *B. pertussis* toxins and cell surface proteins. These vaccines had a greatly improved safety profile compared with wP vaccines [7]. Double-blinded clinical trials and field-efficacy studies for the US licensed aP vaccines estimated the short-term efficacy to be excellent at preventing clinically diagnosed pertussis: 85% after three doses and 98% after five doses [8–10]. Based on these data, the US Advisory Committee on Immunization Practices recommended aP vaccines for all primary and booster doses in 1997 and wP vaccines were phased out in the USA [5].

Pertussis is re-emerging in the USA & other countries

Pertussis was once thought to be a relic of the past and a major success story for worldwide

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vaccination programs. During the wP vaccine era between 1968 and 1992, only 1000–5000 pertussis cases were reported to the CDC annually [11]. However, the USA has experienced a resurgence of pertussis over the past 25 years, beginning roughly at the time of transition from wP to aP vaccines. In each of the last 10 years, greater than 10,000 cases of pertussis have been reported in the USA, and over the same time period, we have seen increasing dramatic peak years of reported pertussis cases in 2004, 2010 and 2012. In 2012, 48,277 cases were reported, the most in nearly 60 years [5]. Since pertussis diagnosis requires patients to become infected and develop symptoms, the actual incidence of *B. pertussis* infection is likely higher than the reported levels of diagnosed pertussis disease.

Among other countries with long-standing aP vaccination programs, not all are experiencing increases in pertussis incidence [12,13]. However, the situation in the USA is mirrored in several developed countries with similar high rates of aP vaccination. Notably, a large outbreak occurred in Australia from 2008 to 2012 when pertussis incidence reached 173 cases per 100,000 people [14] and more than 9000 cases were reported in England in 2012, almost 10-times higher than the number reported in the previous peak year [15].

Why is pertussis resurging?

While the reasons for the pertussis resurgence in the USA and elsewhere are not completely understood, several hypotheses have been proposed [16,17]. The pertussis resurgence is often attributed to vaccine refusal in the USA. However, national data indicate that greater than 95% of toddlers 19–35 months of age have completed the three-dose priming series of aP vaccines arguing against this hypothesis [18].

The correlation between the onset of the pertussis resurgence and the introduction of aP vaccines has led many to hypothesize that they are less effective on a population scale than the wP vaccines they replaced [7,19,20]. Consistent with this notion, several recent observational studies concluded that children primed with aP vaccine had a twofold to fivefold greater risk of being diagnosed with pertussis compared with wP-primed children [21–24].

Another hypothesis as to why pertussis is re-emerging is that the duration of immunity in aP-vaccinated children is shorter than anticipated. Recent cohort and case–control studies concluded that 5 years following the fifth aP dose, children are 4- to 15-fold more likely to acquire pertussis compared with within the first year, consistent with waning aP immunity [10,25–27]. However, it is important to bear in mind that there is not a similar large-scale study tracking duration of immunity in children after wP vaccination so care should be taken in implying a causal relationship between waning immunity elicited by the aP vaccine and the pertussis resurgence.

During the 2012 outbreak in Washington State, greater than 75% of children diagnosed with pertussis were up-to-date on aP vaccinations highlighting that *B. pertussis* can infect fully vaccinated individuals [28]. Another recent study showed that vaccinated patients who are diagnosed with pertussis generally have much milder symptoms compared with unvaccinated people [29]. These findings, combined with data from the baboon model of pertussis discussed below, suggest that aP vaccination prevents symptoms but not infection [30]. Because of this, it is likely that vaccinated people can become asymptomatically infected and, since these people are not diagnosed, the true rate of *B. pertussis* infection in the USA is underreported. Data from the authors' baboon model also raises the intriguing possibility that aP-vaccinated individuals can act as a reservoir for *B. pertussis* circulation in the population [30]. This idea is strengthened by recent seroepidemiological studies showing that *B. pertussis* circulation remains high in countries with excellent aP uptake [31,32]. Considering these data, the authors hypothesized that an underlying cause for the pertussis resurgence is that aP vaccines fail to prevent infection and as a consequence, are less effective at preventing *B. pertussis* circulation within the population compared with wP vaccines [30].

Animal models of pertussis & development of the baboon model

Our ability to counteract the pertussis resurgence is hampered by the fact that pertussis pathogenesis and immunity to natural infection have not been well studied in humans. Although incidence is increasing in some countries, the study of natural pertussis in humans is complicated, because the cyclical nature of pertussis outbreaks and the geographic variability in incidence makes it impossible to predict when or where future outbreaks will occur. 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 (for a review, see: [33]). A variety of animal models have been used to study the pathogenesis of pertussis, including mice, rabbits, guinea pigs and newborn piglets. The strengths and weaknesses of these models have been described in depth previously [34,35]. While these models reproduce many aspects of pertussis, none of them adequately reproduce the full spectrum of the disease observed in humans. For example, the mouse model is widely used to study the innate and adaptive immune responses to pertussis infection and vaccination [19]. However, pertussis-infected mice do not cough or transmit pertussis to naïve mice so these models are not useful for studying the mechanisms of *B. pertussis* circulation or the ability of vaccines to prevent pertussis transmission. Because of their close evolutionary relationship to humans, primates often play a crucial role in research aimed at understanding, preventing and treating infectious diseases in humans. Published studies involving a variety of non-human primate species reported that non-human primates develop all of the characteristic markers of human pertussis [36–41]. In two studies performed in the 1930s, chimpanzees challenged with *B. pertussis* developed severe pertussis characterized by nasopharyngeal colonization and paraxosymal coughing followed by whoop and emesis [40,41]. The peak white blood cell counts in the infected chimpanzees reached 132,000 cells/μl, similar to the levels observed in

severely infected human infants [1,42]. These studies demonstrate that chimpanzees provide an excellent model of pertussis, but ethical considerations preclude their use in pertussis research.

Three studies reported mixed success in infecting Rhesus monkeys with *B. pertussis*. Sauer and Hambrecht challenged 10 Rhesus monkeys (*Macaca mulatta*) and 18 *Cebus capucina* monkeys with *B. pertussis* [39]. Only 3 of 10 Rhesus and 5 of 18 Cebus monkeys developed pertussis. The course of disease in these animals included a mild cough of short duration and two- to threefold rises in white blood cell counts relative to pre-infection levels. Culotta *et al.* challenged 16 Rhesus monkeys and reported mild pertussis symptoms in only 1 animal [36]. When these researchers challenged two *Erythrocebus patas* monkeys, both animals developed pertussis characterized by a four- to fivefold rise in white blood cell counts and a cough illness lasting 28 and 40 days, respectively. In contrast, when Sprunt *et al.* challenged eleven *E. patas* monkeys, all 11 animals were colonized but none developed symptoms [43]. As a first step in developing a reliable non-human primate model of pertussis, the authors sought to revisit the suitability of Rhesus macaques as a model of human clinical pertussis. The authors challenged four young monkeys with *B. pertussis* strain D420, a recent clinical isolate that was isolated from a human infant with severe respiratory distress. Though all four monkeys were colonized, only two had a significant rise in the WBC counts (four- and sixfold, respectively), and just one developed a cough [44]. The results of these studies confirm that pertussis challenge of *M. mulatta* monkeys is not a good model for recapitulating human clinical pertussis. Similarly, the authors and others have found that *Macaca fascicularis* monkeys do not reliably develop pertussis following direct challenge [33]. In contrast, two older studies demonstrated that the closely related species *Macaca cyclopsis* does develop pertussis following challenge [37,38]. In these studies, coughing and animal-to-animal transmission were noted but the disease was mild with peak white blood cell counts noted between 15,000 and 43,000 cells/μl. While these studies suggest that *M. cyclopsis* might provide a good model of pertussis, this species is not readily available for biomedical research.

After unsuccessful attempts at using *M. mulatta* and *M. fascicularis*, the authors hypothesized that the high body temperatures of these animals compared with humans may partly explain the mild disease observed in macaques [33,44]. The authors subsequently found that *B. pertussis* infection of baboons (*Papio anubis*), which have a body temperature closer to humans, produces a disease that is very similar to severe clinical pertussis in children. Upon challenge, baboons experience 4–5 weeks of respiratory colonization and leukocytosis peaking between 30,000 and 80,000 cells/μl, similar to the range in pertussis-infected infants [1,44]. In addition, baboons experience a paroxysmal cough illness characterized by repeated fits of 5–10 coughs. The coughing fits last on average greater than 2 weeks in the baboon, though this is less than some severely infected children, where the cough can last up to 12 weeks [1,44]. We also characterized airborne transmission of *B. pertussis* from infected to naïve animals, which is the route of transmission postulated to occur between humans [45]. Because this is the only model of pertussis to reproduce the cough illness and transmission of the human disease, the authors believe it provides the unique opportunity to study the natural progression of pertussis pathogenesis and host responses to infection and vaccination.

How to use the baboon model effectively: advantages & disadvantages

The baboon model of pertussis has several advantages and disadvantages that need to be considered when designing experiments. Among the major disadvantages is the high cost of biomedical research using primate models. It is also important to understand the ethical considerations related to responsible use of non-human primates [46]. In addition, the baboon model of pertussis requires special facilities for biocontainment since *B. pertussis* is an airborne pathogen. For their experiments, the authors designed a biocontainment bubble with laminar airflow that allows for both direct challenge and airborne transmission studies [45]. The costs and space requirements associated with all primate models necessarily limit the number of experimental groups and replicates that are feasible in any given study. In addition, the number of baboons born each year for biomedical research in the USA is estimated to be about 300, a relatively small number compared with other non-human primate species. Given the requirements to have closely age-matched animals within each experiment, this again limits study sizes. Another drawback is the paucity of immunological reagents that are validated for baboons compared with mice and humans. While antibodies against cell surface markers are generally cross-reactive, anti-cytokine antibodies tend to be much more species-specific in the authors' experience. Finally, there are likely to be genetic and environmental differences between baboons and humans that influence immune responses to vaccination and innate immunity to pertussis infection.

The primary benefit of the baboon model is 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]. In contrast to macaques, baboons express all four IgG subtypes that are found in humans, making them especially suited for vaccine and infectious disease research for a variety of pathogens including respiratory syncytial virus, *Borrelia burgdorferi*, West Nile virus and *Bacillus anthracis* [48–52]. Recent publications have documented the similarity of experimental pertussis in the baboon compared with natural pertussis in humans [44,45]. The baboon model allows researchers to challenge infant baboons in a well-controlled study, providing them the opportunity to answer important questions about pertussis vaccination and pathogenesis that could otherwise not be addressed in small animal models or clinical studies. While small animal models are less expensive and more convenient, their genomes are more divergent with humans. Also, the baboon is the only animal model to demonstrate the paroxysmal cough illness and

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transmission that are characteristics of human pertussis. In human clinical trials, pertussis diagnosis is only made after presentation of symptoms, leading to underreporting of mild or asymptomatic cases [6]. In the baboon model, colonization of the respiratory mucosa and leukocyte counts can be followed serially in baboons to track the infection in an unbiased manner and assess the ability of vaccines to prevent colonization as well as symptoms. These characteristics of the baboon model allow researchers to more easily compare experimental groups than in human clinical trials making the baboon a very useful model for pre-clinical testing of novel pertussis vaccines and therapies.

What the baboon model can teach us?

Using the baboon model to study current vaccines and as a pre-clinical model for characterizing novel vaccines

In a recent study, the authors sought to determine if pertussis vaccination and immunity from previous infection are able to prevent *B. pertussis* colonization and transmission (summarized in Table 1) [30]. Using the adolescent baboon model, animals were vaccinated with licensed diphtheria, tetanus and pertussis vaccines containing either wP or aP components at 2, 4 and 6 months of age and challenged at 7 months (see Table 2 for a description of the vaccines used). The aP group consisted of equal numbers of animals vaccinated with Daptacel® or Infanrix®.

When directly challenged with a clinical isolate of *B. pertussis*, unvaccinated animals were heavily colonized and had high leukocytosis before clearing the infection approximately 30 days following challenge. Convalescent (previously infected) animals were not colonized and had no leukocytosis suggesting that the adaptive immune response induced by pertussis infection prevents re-infection. Vaccination with wP provided intermediate protection: the animals were colonized but had no leukocytosis and cleared the infection approximately 2 weeks faster than unvaccinated animals. Animals vaccinated with aP were similarly protected from leukocytosis but were heavily colonized and did not clear the infection faster than unvaccinated animals. The authors also tested whether aP could prevent infection following airborne transmission from an challenged animal and found there was no difference in the peak levels of colonization or kinetics of colonization in the aP-vaccinated and -unvaccinated animals infected by transmission. These data suggest that in baboons, aP vaccines are highly effective at preventing symptoms following pertussis infection. This is an important point since the degree of leukocytosis correlates with rates of hospitalization and fatality following pertussis infection in infants [42]. This is also consistent with a recent report that pertussis disease is less severe in patients who had previously received aP vaccination compared with patients who had not been vaccinated [29]. However, if the authors' findings are relevant to humans, these data suggest that vaccinated people can be asymptomatically infected with *B. pertussis* and act as a reservoir for circulation within communities. To test this hypothesis in baboons, the authors vaccinated the animals with aP and directly challenge them. Each vaccinated animal was placed in a separate cage and co-housed with an unchallenged animal. In each case, the unchallenged animals gradually became infected and attained high levels of colonization. These data confirm that it is possible for aP-vaccinated baboons to transmit *B. pertussis* even when asymptomatic.

In an effort to understand how differences in the immune responses to pertussis infection and vaccination may correlate with the dramatic differences in the ability of each to prevent colonization, the authors analyzed serum antibody levels and T-cell skewing in the different groups prior to challenge. The authors observed no major differences in antibody titers to the four vaccine antigens or to whole-cell *B. pertussis* between the aP, wP and convalescent groups suggesting that while aP-induced antibody responses are likely sufficient for preventing symptoms, they cannot prevent mucosal colonization. This notion is further supported by an observation the authors made in their initial characterization of the model: when convalescent baboons were re-challenged, the authors did not observe boosting of anti-pertussis titers until day 5 following the secondary challenge [44]. Since these animals were protected from colonization prior to the observed antibody boosting, these data suggest that T-cell memory plays a substantial role in the protective immunity observed in convalescent baboons. The authors characterized the primary immune response to pertussis infection by quantifying mucosal cytokine expression before and after infection. Infected animals had strong induction of IL-6, IL-23 and transient IL-1β [53]. Consistent with the role of these cytokines in the development of IL-17-producing T cells (Th17 cells), the authors also observed strong expression of IL-17 at the mucosa. In addition, several cytokines and chemokines that are orchestrated by Th17 immune responses were detected. The induction of IL-17 and IFN-γ-secreting $CD4^+$ cells in convalescent animals was also observed, consistent with the induction of adaptive Th17 and Th1 responses. Importantly, these cells still remained 2 years after infection, suggesting the T-cell memory to pertussis infection is long-lived. These data shed important light on the innate and adaptive immune responses to pertussis in a primate infection model and suggest that Th17 and Th1 immune responses contribute to the sterilizing immunity conferred by *B. pertussis* infection. When animals were vaccinated with aP or wP, the authors found different T-cell skewing, depending on the vaccine. For the wP-vaccinated animals, the authors observed similar Th17 and Th1 skewing, though the responses were milder than infection. The aP-vaccinated animals on the other hand displayed a mixed Th2 and Th1 immune response with no indication of Th17 immunity. From these data it appears that Th17 responses correlate with protection against *B. pertussis* infection since greater Th17 responses were associated with the more protective immune responses elicited by previous infection, and to a lesser degree, wP vaccination. Two recent studies in children vaccinated with Infanrix confirmed the authors' finding that aP induces Th2 and Th1 responses with no Th17 response [54,55]. To date, Th17 responses have not been

Table 1. Summary of pertussis vaccine studies in the baboon model.

Study design	Immune status	Age at challenge (number of animals)	Outcome	Ref.
Adolescent challenge	Naïve	4–10 months (13)	Animals were visibly ill, colonized for 4–5 weeks and developed cough illness and high leukocytosis. Mucosal cytokine responses in the first 2 weeks were characterized by high IL-6 as well as IL-17 and other cytokines and chemokines associated with IL-17 responses. Antibody titers were detectable by 2–3 weeks, peaked around the time of clearance and remained high for at least 6 months. Following clearance, animals developed long-lived (>2 years) Th17 and Th1 memory responses	[30,44,53]
	aP vaccinated at 2, 4, 6 months	7 months (4)	Antibody titers were very high prior to challenge and animals possessed Th2 and Th1 memory responses. Animals were colonized for 4–5 weeks but did not cough or have leukocytosis	[30]
	wP vaccinated at 2, 4, 6 months	7 months (3)	Antibody titers were very high prior to challenge and animals possessed Th17 and Th1 memory responses. Animals were colonized for 2–3 weeks but did not cough or have leukocytosis	[30]
	Convalescent (previously challenged animals that naturally cleared the infection)	7–10 months at re-challenge (8)	Antibody titers were very high prior to challenge and animals possessed stronger Th17 and Th1 immune memory responses compared to wP vaccination. Following second challenge, animals were not colonized and had no symptoms	[30,44,53]
Infant challenge	Naïve	5 weeks (3)	Animals were severely ill with cough illness and high leukocytosis. Complications included reduced physical activity (2/3) and death related to bacterial pneumonia (1/3). Animals were colonized for about 5 weeks	[86]
	Neonatal vaccination: aP vaccinated at 2 days	5 weeks (2)	Animals possessed significant anti-pertussis antibody titers prior to infection. Upon challenge, animals were colonized for 5–6 weeks but appeared healthy and did not cough or have leukocytosis	[86]
	Neonatal vaccination: aP vaccinated at 2, 28 days	5 weeks (2)	Anti-pertussis antibody titers prior to infection were higher compared to the single vaccination group. Upon challenge, animals were colonized for 5–6 weeks but appeared healthy and did not cough or have leukocytosis	[86]
	Maternal vaccination (mother primed with aP prior to pregnancy and boosted with aP during the third trimester)	5 weeks (7)	Animals had significant anti-pertussis antibody titers prior to infection. Upon challenge, animals were colonized for 5–6 weeks but appeared healthy and did not cough or have leukocytosis	[86]

aP: Acellular pertussis; wP: Whole-cell pertussis.

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Table 2. Components of pertussis vaccines used in baboon studies.

	aP[†]		wP
Trade name	Daptacel®	Infanrix®	Triple Antigen
Manufacturer	Sanofi Pasteur, Ltd.	GlaxoSmithKline Biologicals	Serum Institute of India
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

[†]For all reported baboon studies, the aP group consisted of equal numbers of animals vaccinated with Daptacel or Infanrix.
aP: Acellular pertussis; Lf: Limit of flocculation; IU: International unit; wP: Whole-cell pertussis.

studied in wP-vaccinated humans or pertussis-infected humans. A potential protective role for Th17 memory is intriguing since these responses have been shown to be important in controlling extracellular bacterial infections at respiratory surfaces through the induction of granulopoiesis and recruitment of neutrophils and macrophages to the infected mucosa [56]. Previous work in mouse models has shown that IL-17 signaling is important for protection from *B. pertussis* and other extracellular respiratory pathogens, including *Klebsiella pneumoniae* [57–60]. Further data will be required to determine what role IL-17 and Th17 cells play in protection from *B. pertussis* in baboons and whether a similar response is observed in infected humans.

In the future, this same experimental outline can be applied for studying the ability of novel vaccines to prevent colonization and transmission. A major lingering question in pertussis vaccine research is whether it is possible to skew the immune response toward more protective cell-mediated immunity by substituting novel adjuvants for the aluminum phosphate adjuvant used in currently licensed vaccines [61]. Current data on the wP vaccine suggests that inducing a more natural immune response is likely to result in greater protection. Data from the mouse model suggest that addition of the toll-like receptor 9 (TLR9) agonist CpG to alum-adjuvanted aP vaccines enhances Th1 immunity and provides better protection from colonization compared with aP vaccines with alum only [58,62]. Since TLR4 appears to be critical for innate recognition of *B. pertussis*, it seems plausible that an aP vaccine combined with a TLR4 agonist (e.g., the lipopolysaccharide derivative monophosphoryl lipid A) could induce an immune response that more closely resembles the protective responses to infection or wP vaccination while retaining the exceptional safety profile of the current aP vaccines. Such a vaccine could be particularly useful as a booster if it is able to 'reprogram' the Th2 immune response induced by the infant primary aP vaccine doses and push the immune response towards a more protective immune response. The baboon model will also be extremely useful in testing novel routes of immunization. Given the compartmentalization of the mucosal immune system, it is possible that a properly adjuvanted aP or live, attenuated vaccine administered intranasally would provide much better protection from colonization than the same vaccine administered parenterally. Data from human cell culture and mouse models suggest that intranasal administration of the live, attenuated pertussis vaccine, BPZE1, induces a more natural immune response characterized by Th17 and Th1 immune responses [63,64].

Because it allows researchers to assess vaccine-mediated protection through direct challenge, the baboon model will also be useful in defining immunological correlates that predict protection against *B. pertussis* colonization and pertussis symptoms. Immunologic correlates provide a major advantage in characterizing vaccines because they allow efficacy to be determined using a validated laboratory assay in place of much more costly clinical end point efficacy studies [65]. In most diseases where an immunological correlate has been defined, protection is predicted by a certain level of serum antibody to a specific antigen. For example, protection from tetanus requires vaccinees to have a certain level of serum anti-tetanus toxin. For other diseases that require cell-mediated immunity for protection, it has been proposed that cytokine responses and T-cell memory profiles may provide more relevant immunological correlates [66]. The fact that *B. pertussis* infection provides immunity to re-infection in the baboon model argues for the existence of a strong and protective adaptive immune response [44,53]. However to date, there is no scientifically well-established immunologic marker that predicts protection against pertussis [67]. This is likely because of the complexities of the host immune responses required to prevent both colonization at the respiratory mucosa and systemic toxin-mediated symptoms. It is likely that serum antibody levels against pertussis toxin are required for preventing severe pertussis, at least in part by blocking pertussis-toxin induced leukocytosis [68,69]. However, it is possible that Th17 and/or

Th1 memory responses may provide a correlate for prevention of *B. pertussis* colonization [30].

Identifying vaccine strategies to protect newborns

The highest incidence of pertussis is in infants too young to have completed primary vaccinations and the vast majority of severe pertussis cases requiring hospitalization and fatal cases occur in infants less than 3 months of age [3,4]. In infants requiring admission to intensive care units, mortality rates can approach 50–70% and common complications include bacterial pneumonia and pulmonary hypertension [42,70–72]. These under-vaccinated infants also display the highest levels of leukocytosis following pertussis infection and it is postulated that the high leukocyte mass can lead to vessel blockage, restricted blood flow and exacerbate the development of pulmonary hypertension [73].

Several strategies have been proposed to reduce the incidence of pertussis in infants during the first few months of life, including cocooning, neonatal vaccination and maternal vaccination. Cocooning was proposed as a mechanism to indirectly reduce infant exposure to *B. pertussis* by vaccinating parents, siblings and other frequent contacts of newborns. The rationale for cocooning is based on the evidence that infants most often acquire pertussis from a parent or other family member [74–76]. However, in an evaluation of the effectiveness of cocooning in four Houston hospitals, no significant benefit was observed [77]. Another disadvantage of cocooning is that it is not cost–effective compared with strategies that provide direct protection to the newborn [78]. Additionally, the authors recently showed that aP vaccinated baboons are not protected from *B. pertussis* infection and can transmit pertussis to naïve cage mates, suggesting that cocooning may not optimally protect infants even if fully implemented [30]. The apparent failures to reduce infant pertussis by vaccinating contacts suggest that newborns need anti-pertussis antibodies in order to be protected for the first few months of life. Immunization at birth (neonatal vaccination) has been proposed based on the demonstration that newborns are able to mount antibody responses to aP vaccination [79,80]. Alternatively, vaccinating women in the third trimester of pregnancy has been proposed and is now recommended by the US Advisory Committee on Immunization Practices [81,82]. Several studies demonstrated safety and efficient transplacental transfer of anti-pertussis antibodies supporting the potential of this approach [83–85]. However, data showing that either strategy is effective at reducing severe pertussis in infants has not been available. Using their infant baboon model, the authors evaluated the ability of neonatal and maternal vaccination to protect neonates against severe disease following direct challenge with *B. pertussis* [86]. The maternal and neonatal vaccination groups consisted of equal numbers of animals vaccinated with Daptacel and Infanrix. For the maternal vaccination study, eight adult female baboons were primed with aP vaccine. After becoming pregnant, the animals were boosted with the same aP at the beginning of the third trimester. Seven infants born to vaccinated mothers and three infants born to unvaccinated mothers were weaned at 4 weeks of age and challenged a week later. All three infants born to unvaccinated mothers were noticeably more ill compared with pertussis-infected adolescent baboons and exhibited severe pertussis characterized by respiratory colonization, high leukocytosis and cough illness. Two of the three animals had reduced physical activity and one died from complications from bacterial pneumonia. The infants born to aP-vaccinated mothers had equivalent levels of *B. pertussis* colonization but possessed anti-pertussis serum antibodies and were fully protected from all signs and symptoms of severe pertussis. In agreement with these findings, recent data from the UK suggest that the large-scale implementation of a maternal vaccination program reduced severe pertussis in infants during the 2012 outbreak. When pregnant women received a tetanus diphtheria and acellular pertussis booster at least 7 days prior to delivery, maternal vaccination was 91% effective at preventing hospitalization of infants due to severe pertussis [13].

For the neonatal vaccination study, two animals were vaccinated with a single aP dose at 2 days of age and two additional animals were vaccinated twice, at 2 days and 28 days. A single aP vaccination at 2 days of age resulted in increased anti-pertussis antibody titers with some boosting observed in the group receiving two aP vaccinations. Following direct challenge with *B. pertussis* at 5 weeks of age, all four neonatal vaccinated animals were heavily colonized but fully protected from severe pertussis signs and symptoms. Similar to the authors' results in 7-month-old baboons, these data suggest that it is possible for the immune response to aP to prevent severe pertussis without blocking respiratory colonization. While there are no clinical efficacy data on the ability of neonatal vaccination to prevent pertussis, these findings are consistent with recent studies showing that aP vaccines reduce pertussis morbidity and hospitalization rates in infants after a single dose at 2–3 months of age [87,88].

The fact that protection was equivalent in neonatal vaccinated infants and maternal vaccinated infants suggests that aP-induced antibody responses are sufficient to prevent leukocytosis and severe pertussis. The authors' results in the baboon model provide a proof-of-concept that both neonatal and maternal vaccination can confer protection against severe pertussis during the first months of life. For neonatal vaccination, it is important to consider that there is a delay between vaccination and the induction of protective immunity. So although neonatal vaccination is likely to shorten the window of vulnerability, infants will remain vulnerable in the initial weeks following the birth dose of vaccine. In addition, maternal vaccination provides the added advantages of protecting the mother as well as providing antibodies to the infant from birth. Another important consideration is that neonatal vaccination seems to be more likely to interfere with future vaccinations in the infants compared with maternal vaccination [89,90]. For these reasons, maternal vaccination has recently gained favor in the USA and other countries [6]. The infant baboon model can provide an efficient and cost–effective approach to obtain protection data when exploring different combinations of priming

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and boosting vaccines for use in maternal vaccination in the developed and developing world and for evaluating the ability of novel vaccines to protect newborns. In addition, this model will allow researchers to further investigate the mechanisms that contribute to fatal pertussis disease in newborns and test the effectiveness of novel therapeutics or critical care strategies aimed at reducing mortality from severe pertussis.

Using the baboon model as a tool to study pertussis pathogenesis & identify novel antigens

Because of the dramatic cough associated with classical pertussis, the well-established dogma is that *B. pertussis* infection is transmitted via aerosolized respiratory droplets [1]. However, since cough and transmission are not observed in any of the animal models, it was never possible to definitively characterize the route of *B. pertussis* transmission. In the baboon model, infected animals experience a significant paroxysmal cough illness that lasts for at least 2 weeks when the animal is most heavily colonized [44]. The authors next designed an experiment to ask whether *B. pertussis* is transmissible in baboons. Two animals were directly challenged with *B. pertussis* and were co-housed with two unchallenged animals to study *B. pertussis* transmission between baboons in close contact. In addition, animals were placed in cages seven feet away from the infected animals in such a way that these animals could only become infected by airborne transmission [45]. The authors found that all co-housed animals became colonized after an average of 9 days. Colonization in the transmission animals increases gradually and reaches levels that are identical to those seen in directly challenged animals. Animals in the isolated cages also became infected after an average of 19 days suggesting that the force of infection decreases with distance from the shedding animals. The authors believe the baboon model will be instrumental in furthering the understanding of pertussis transmission through elucidation of the mechanisms and pathogen and host factors involved. Another important use of the baboon model will be studying the mechanisms of the cough and the link between cough and transmission. Currently, the mechanism by which *B. pertussis* infection induces the very unique paroxysmal cough is a subject of much controversy [91]. It is important to note that the authors observed transmission from aP-vaccinated animals in the absence of coughing suggesting that cough is not absolutely required for transmission. In addition, since aP-vaccinated baboons are protected from cough in spite of high levels of respiratory colonization, these data suggest that anti-pertussis serum antibodies in vaccinated animals can prevent the cough illness. This is also consistent with the recent finding that pertussis cases in vaccinated individuals are less severe and are less likely to be associated with severe cough illness compared with unvaccinated people [29].

The baboon model will also be useful for asking targeted questions about pertussis pathogenesis [91,92]. As an example, since the amount of adenylate cyclase toxin produced during an infection is unknown, it is not clear what role the toxin plays in pathogenesis and what concentrations of toxin are relevant to use *in vitro*. Eby *et al*. showed that adenylate cyclase toxin concentrations in the nasopharyngeal washes from baboons at the peak of infection ranged from 1 to 5 ng/ml [93]. This was similar to the levels characterized in samples from two human infants (12 and 20 ng/ml). Bearing in mind that these concentrations are diluted during the nasopharyngeal wash procedure, these data confirm that adenylate cyclase toxin is expressed in significant amounts during *B. pertussis* infection and suggest a relevant range of toxin concentrations that can be used for *in vitro* studies.

There are several long-standing questions with regards to pertussis pathogenesis that the baboon model will be useful in addressing. For example, the only studies examining the sites of *B. pertussis* colonization in the respiratory tract and pertussis-associated pathology in humans comes from autopsy examinations of pertussis-infected infants [1,72]. Since pertussis fatality rates are at most 2% for the youngest infants, these data represent the most severe pertussis cases. The baboon model can be used to further the understanding of pertussis pathology during normal, uncomplicated pertussis infection in primates by collecting samples at various sites in the respiratory tract and analyzing for colonization and histopathology. Similar studies will also be useful for determining whether vaccination causes differences in colonization patterns in vaccinated versus unvaccinated baboons.

The authors also believe the baboon model will allow researchers to identify novel virulence factors that are required for pertussis infection and could potentially be developed as vaccine antigens. Alternatively, potential virulence factors that are identified *in vitro* or in small animal models can be confirmed in the baboon model. The importance of this type of discovery research is highlighted by the emergence of pertactin-deficient *B. pertussis* strains over the last several years in the USA and elsewhere. In 2012, these strains were estimated to make up >50% of the circulating strains in the USA [94,95]. Pertactin-deficient strains are appearing simultaneously in other countries that use aP, possibly due to the rapid global transmission of *B. pertussis* strains [96–99]. While pertactin is considered a protective antigen, there do not appear to be any major clinical differences between patients infected with pertactin-deficient or pertactin-expressing strains [100]. Consistent with these findings, a pertactin-deficient clinical isolate obtained from the CDC was tested in a preliminary study in the baboon model and the authors observed no defects in colonization or leukocytosis compared with a clinical isolate expressing pertactin [Warfel JM, Merkel TJ, Unpublished Data]. Further, deletion of the pertactin-encoding gene from *B. pertussis* causes only minor if any deficiencies in lung colonization in the mouse model arguing against the requirement for compensatory mutations in the pertactin-deficient strains [101]. So while pertactin may be protective against wild-type strains, these data suggest it is not required for *B. pertussis* infection, which has likely contributed to the rapid evolution of pertactin-deficient strains. In the future, the baboon model will be useful in identifying essential virulence factors that are immunogenic that would be strong candidates for next-generation vaccines. Finally, the baboon model provides a powerful tool for testing the assumptions

guiding next-generation pertussis vaccines and for generating proof-of-concept to support clinical trials of new vaccines.

Expert commentary

In spite of widespread vaccine coverage, pertussis is re-emerging in the USA and other developed countries. Our incomplete understanding of pertussis pathogenesis and vaccinology has hampered our ability to respond to this important public health concern. Many questions remain unanswered because pertussis is not well studied in humans and, until recently, there has not been an animal model that adequately reproduces the full spectrum of the human disease. Experimental pertussis in the recently developed baboon model closely matches clinical pertussis in humans, including the development of paroxysmal cough and the demonstration of host-to-host transmission. The baboon model is a powerful tool with enormous potential to unravel many of the long-standing mysteries of pertussis pathogenesis and identify the mechanisms underlying the worldwide pertussis resurgence. In the last 2 years, data from the baboon model have already provided the first definitive evidence that *B. pertussis* is transmitted by respiratory droplets, have vastly increased our knowledge of immunity to *B. pertussis* infection and vaccination and have suggested that aP vaccines control pertussis symptoms but do not prevent colonization or transmission. In addition, the baboon model has provided proof-of-concept that maternal and neonatal vaccination will be effective at preventing infant pertussis. These results, summarized in Table 1, highlight the utility of the baboon model as an important tool for pre-clinical evaluation of pertussis vaccines and therapeutics.

Five-year view

In the next 5 years, the reagent tool kit available for characterization of baboon immune responses will be greatly improved, allowing us to probe even deeper into the complex host–pathogen interactions. Data from the baboon model will uncover potential antigens for novel aP vaccines by identifying the immune targets to infection, which is more protective than vaccination. Pre-clinical studies testing novel vaccine adjuvants and routes of administration will provide a solid immunological basis to begin to develop novel vaccines that are capable of preventing colonization and transmission of *B. pertussis*.

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Key issues

- Despite widespread vaccine coverage in the USA, pertussis incidence has been increasing at an alarming rate.
- The lack of an animal model that fully replicates the human disease has limited our understanding of pertussis pathogenesis and hampered our ability to respond to this important public health concern.
- A baboon model for pertussis was recently developed that closely matches the clinical illness, including development of paroxysmal cough and demonstration of host-to-host transmission by aerosolized respiratory droplets.
- Acellular pertussis vaccination in baboons induces a Th2-skewed immune response and prevents pertussis-associated symptoms but does not prevent mucosal colonization or host-to-host transmission.
- Infection by live *Bordetella pertussis* and whole-cell vaccination induce immune responses that are characterized by Th17 memory and provide protection from colonization.
- These data suggest that acellular-vaccinated people are a possible reservoir for *B. pertussis* circulation and that Th17 responses may be required to prevent *B. pertussis* colonization.
- In the future, the baboon model will be useful for unraveling some of the mysteries of pertussis pathogenesis and as a pre-clinical tool for evaluating novel pertussis vaccines.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. Clin Microbiol Rev 2005;18(2):326-82
2. Heininger U. Update on pertussis in children. Expert Rev Anti Infect Ther 2010;8(2):163-73
3. Haberling DL, Holman RC, Paddock CD, Murphy TV. Infant and maternal risk factors for pertussis-related infant mortality in the United States, 1999 to 2004. Pediatr Infect Dis J 2009;28(3):194-8
4. Winter K, Harriman K, Zipprich J, et al. California pertussis epidemic, 2010. J Pediatr 2012;161(6):1091-6
5. Clark TA. Changing pertussis epidemiology: everything old is new again. J Infect Dis 2014;209(7):978-81
6. Libster R, Edwards KM. Re-emergence of pertussis: what are the solutions? Expert Rev Vaccines 2012;11(11):1331-46
7. Zhang L, Prietsch SO, Axelsson I, Halperin SA. Acellular vaccines for preventing whooping cough in children.

doi: 10.1586/14760584.2014.946016

Cochrane Database Syst Rev 2012;3: CD001478

- **Comprehensive review of the safety and efficacy data from the acellular pertussis (aP) vaccine trials. Metadata analysis suggests that aP vaccines containing 3–5 antigens are more effective than those containing 1 or 2 antigens.**

8. Greco D, Salmaso S, Mastrantonio P, et al. A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. Progetto Pertosse Working Group. N Engl J Med 1996;334(6):341-8
9. Gustafsson L, Hallander HO, Olin P, et al. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. N Engl J Med 1996;334(6):349-55
10. Misegades LK, Winter K, Harriman K, et al. Association of childhood pertussis with receipt of 5 doses of pertussis vaccine by time since last vaccine dose, California, 2010. JAMA 2012;308(20):2126-32

- **Demonstrated the waning of immunity following the 5th dose of aP vaccine during the 2010 pertussis outbreak in California.**

11. CDC. Pertussis: surveillance and reporting. 2014. Available from: www.cdc.gov/pertussis/surv-reporting.html
12. Jackson DW, Rohani P. Perplexities of pertussis: recent global epidemiological trends and their potential causes. Epidemiol Infect 2013;1-13
13. WHO. SAGE Pertussis Working Group Background paper. 2014. Available from: www.who.int/entity/immunization/sage/meetings/2014/april/1_Pertussis_background_FINAL4_web.pdf?ua=1
14. Australian Government Department of Health. National notifiable diseases surveillance system. 2014. Available from: www9.health.gov.au/cda/source/cda-index.cfm
15. Public Health England. Laboratory confirmed cases of Pertussis infection by laboratory method, year and quarter, England: 2002-2013. 2014. Available from: www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133571358
16. Friedrich MJ. Research aims to boost pertussis control. JAMA 2011;306(1):27-9
17. Poland GA. Pertussis outbreaks and pertussis vaccines: new insights, new concerns, new recommendations? Vaccine 2012;30(49):6957-9
18. CDC. National, state, and local area vaccination coverage among children aged 19-35 months – United States, 2011. Morb Mortal Wkly Rep 2012;61:689-96
19. Higgs R, Higgins SC, Ross PJ, Mills KH. Immunity to the respiratory pathogen Bordetella pertussis. Mucosal Immunol 2012;5(5):485-500
20. Cherry JD. Epidemic pertussis in 2012 – the resurgence of a vaccine-preventable disease. N Engl J Med 2012;367(9):785-7
21. Witt MA, Arias L, Katz PH, et al. Reduced risk of pertussis among persons ever vaccinated with whole cell pertussis vaccine compared to recipients of acellular pertussis vaccines in a large US cohort. Clin Infect Dis 2013;56(9):1248-54
22. Liko J, Robison SG, Cieslak PR. Priming with whole-cell versus acellular pertussis vaccine. N Engl J Med 2013;368(6):581-2
23. Sheridan SL, Ware RS, Grimwood K, Lambert SB. Number and order of whole cell pertussis vaccines in infancy and disease protection. JAMA 2012;308(5):454-6

- •• **Compared the relative risk of pertussis diagnosis among children who received either all whole-cell pertussis primary vaccinations, all aP primary vaccinations or a mixed schedule in the 1998 birth cohort in Queensland, Australia.**

24. Klein NP, Bartlett J, Fireman B, et al. Comparative effectiveness of acellular versus whole-cell pertussis vaccines in teenagers. Pediatrics 2013;131(6):e1716-22
25. Klein NP, Bartlett J, Rowhani-Rahbar A, et al. Waning protection after fifth dose of acellular pertussis vaccine in children. N Engl J Med 2012;367(11):1012-19
26. Tartof SY, Lewis M, Kenyon C, et al. Waning immunity to pertussis following 5 doses of DTaP. Pediatrics 2013;131(4): e1047-52
27. Witt MA, Katz PH, Witt DJ. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. Clin Infect Dis 2012;54(12): 1730-5
28. CDC. Pertussis epidemic – Washington, 2012. MMWR Morb Mortal Wkly Rep 2012;61(28):517-22
29. Barlow RS, Reynolds LE, Cieslak PR, Sullivan AD. Vaccinated children and adolescents with pertussis infections experience reduced illness severity and duration, Oregon, 2010-2012. Clin Infect Dis 2014;58(11):1523-9
30. Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci USA 2014;111(2): 787-92

- •• **Compared the immune responses with whole-cell pertussis vaccination, aP vaccination and previous pertussis infection, and characterized the ability of each to prevent *Bordetella pertussis* colonization and symptoms. Demonstrated that aP vaccinated baboons can be infected with *B. pertussis* and transmit pertussis to contacts.**

31. de Greeff SC, de Melker HE, van Gageldonk PG, et al. Seroprevalence of pertussis in The Netherlands: evidence for increased circulation of Bordetella pertussis. PLoS One 2010;5(12):e14183
32. Lai FY, Thoon KC, Ang LW, et al. Comparative seroepidemiology of pertussis, diphtheria and poliovirus antibodies in Singapore: waning pertussis immunity in a highly immunized population and the need for adolescent booster doses. Vaccine 2012; 30(24):3566-71
33. Merkel TJ, Halperin SA. Nonhuman primate and human challenge models of pertussis. J Infect Dis 2014;209(Suppl 1): S20-3
34. van der Ark AA, Hozbor DF, Boog CJ, et al. Resurgence of pertussis calls for re-evaluation of pertussis animal models. Expert Rev Vaccines 2012;11(9):1121-37
35. Elahi S, Holmstrom J, Gerdts V. The benefits of using diverse animal models for studying pertussis. Trends Microbiol 2007; 15(10):462-8
36. Culotta C, Harvey D, Gordon E. Whooping cough II: experimental study. J Pediatr 1935;6:743-52
37. Huang CC, Chen PM, Kuo JK, et al. Experimental Whooping cough. N Engl J Med 1962;266:105-11
38. Lin T. Experimental whooping cough in monkey. J Formos Med Ass 1958;57:53-62
39. Sauer L, Hambrecht L. Experimental whooping cough. Am J Dis Child 1929;37: 732-44
40. Rich AR, Long PH, Brown JH, et al. Experiments upon the cause of whooping cough. Science 1932;76(1971):330-1
41. Shibley G. Etiology of whooping cough. Proc Soc Exp Biol Med 1934;31:576-9
42. Berger JT, Carcillo JA, Shanley TP, et al. Critical pertussis illness in children: a multicenter prospective cohort study. Pediatr Crit Care Med 2013;14(4):356-65
43. Sprunt DH, Martin DS, McDearman S. Results of the intratracheal injection of the

bordet-gengou bacillus in the monkey and rabbit. J Exp Med 1938;67(2):309-22

44. Warfel JM, Beren J, Kelly VK, et al. Nonhuman primate model of pertussis. Infect Immun 2012;80(4):1530-6

45. Warfel JM, Beren J, Merkel TJ. Airborne transmission of Bordetella pertussis. J Infect Dis 2012;206(6):902-6

46. Tardif SD, Coleman K, Hobbs TR, Lutz C. IACUC review of nonhuman primate research. ILAR J 2013;54(2):234-45

47. Perry DL, Bollinger L, White GL. The Baboon (Papio spp.) as a model of human Ebola virus infection. Viruses 2012;4(10): 2400-16

48. Shearer MH, Dark RD, Chodosh J, Kennedy RC. Comparison and characterization of immunoglobulin G subclasses among primate species. Clin Diagn Lab Immunol 1999;6(6):953-8

49. Hefty PS, Brooks CS, Jett AM, et al. OspE-related, OspF-related, and Elp lipoproteins are immunogenic in baboons experimentally infected with Borrelia burgdorferi and in human lyme disease patients. J Clin Microbiol 2002;40(11):4256-65

50. Wolf RF, Papin JF, Hines-Boykin R, et al. Baboon model for West Nile virus infection and vaccine evaluation. Virology 2006; 355(1):44-51

51. Papin JF, Wolf RF, Kosanke SD, et al. Infant baboons infected with respiratory syncytial virus develop clinical and pathological changes that parallel those of human infants. Am J Physiol Lung Cell Mol Physiol 2013;304(8):L530-9

52. Stearns-Kurosawa D, Lupu F, Taylor FJ, et al. Sepsis and pathophysiology of anthrax in a nonhuman primate model. Am J Pathol 2006;169(2):433-44

53. Warfel JM, Merkel TJ. Bordetella pertussis infection induces a mucosal IL-17 response and long-lived Th17 and Th1 immune memory cells in nonhuman primates. Mucosal Immunol 2013;6(4):787-96

- **Characterized the innate and adaptive immune responses to *B. pertussis* infection in baboons. Identified Th17 and Th1 memory populations that persist over 2 years after infection.**

54. Schure RM, Hendrikx LH, de Rond LG, et al. T-cell responses before and after the fifth consecutive acellular pertussis vaccination in 4-year-old Dutch children. Clin Vaccine Immunol 2012;19(11): 1879-86

55. Schure RM, Hendrikx LH, de Rond LG, et al. Differential T- and B-cell responses to pertussis in acellular vaccine-primed versus whole-cell vaccine-primed children 2 years after preschool acellular booster vaccination. Clin Vaccine Immunol 2013;20(9):1388-95

56. Kolls JK, Khader SA. The role of Th17 cytokines in primary mucosal immunity. Cytokine Growth Factor Rev 2010;21(6):443-8

57. Ye P, Garvey PB, Zhang P, et al. Interleukin-17 and lung host defense against Klebsiella pneumoniae infection. Am J Respir Cell Mol Biol 2001;25(3):335-40

58. Ross PJ, Sutton CE, Higgins S, et al. Relative contribution of Th1 and Th17 cells in adaptive immunity to Bordetella pertussis: towards the rational design of an improved acellular pertussis vaccine. PLoS Pathog 2013;9(4):e1003264

59. Zhang X, Goel T, Goodfield LL, et al. Decreased leukocyte accumulation and delayed Bordetella pertussis clearance in IL-6-/- mice. J Immunol 2011;186(8): 4895-904

60. Andreasen C, Powell DA, Carbonetti NH. Pertussis toxin stimulates IL-17 production in response to Bordetella pertussis infection in mice. PLoS One 2009;4(9):e7079

61. Plotkin SA. The pertussis problem. Clin Infect Dis 2014;58(6):830-3

62. Asokanathan C, Corbel M, Xing D. A CpG-containing oligodeoxynucleotide adjuvant for acellular pertussis vaccine improves the protective response against Bordetella pertussis. Hum Vaccin Immunother 2013;9:2

63. Fedele G, Bianco M, Debrie AS, et al. Attenuated Bordetella pertussis vaccine candidate BPZE1 promotes human dendritic cell CCL21-induced migration and drives a Th1/Th17 response. J Immunol 2011;186(9):5388-96

64. Feunou PF, Bertout J, Locht C. T- and B-cell-mediated protection induced by novel, live attenuated pertussis vaccine in mice. Cross protection against parapertussis. PLoS One 2010;5(4):e10178

65. Farizo KM, Burns DL, Finn TM, et al. Clinical evaluation of pertussis vaccines: US Food and Drug Administration regulatory considerations. J Infect Dis 2014; 209(Suppl 1):S28-31

66. Plotkin SA. Complex correlates of protection after vaccination. Clin Infect Dis 2013;56(10):1458-65

67. Meade BD, Plotkin SA, Locht C. Possible options for new pertussis vaccines. J Infect Dis 2014;209(Suppl 1):S24-7

68. Taranger J, Trollfors B, Lagergård T, et al. Correlation between pertussis toxin IgG antibodies in postvaccination sera and subsequent protection against pertussis. J Infect Dis 2000;181(3):1010-13

69. Cherry JD, Gornbein J, Heininger U, Stehr K. A search for serologic correlates of immunity to Bordetella pertussis cough illnesses. Vaccine 1998;16(20):1901-6

70. Namachivayam P, Shimizu K, Butt W. Pertussis: severe clinical presentation in pediatric intensive care and its relation to outcome. Pediatr Crit Care Med 2007;8(3): 207-11

71. Halasa NB, Barr FE, Johnson JE, Edwards KM. Fatal pulmonary hypertension associated with pertussis in infants: does extracorporeal membrane oxygenation have a role? Pediatrics 2003;112(6 Pt 1):1274-8

72. Paddock CD, Sanden GN, Cherry JD, et al. Pathology and pathogenesis of fatal Bordetella pertussis infection in infants. Clin Infect Dis 2008;47(3):328-38

73. Ulloa-Gutierrez R, Boza R, Carvajal-Riggioni D, Baltodano A. Pertussis: should we improve intensive care management or vaccination strategies? Expert Rev Vaccines 2011;10(1):49-53

74. Bisgard KM, Pascual FB, Ehresmann KR, et al. Infant pertussis: who was the source? Pediatr Infect Dis J 2004;23(11):985-9

75. Kowalzik F, Barbosa AP, Fernandes VR, et al. Prospective multinational study of pertussis infection in hospitalized infants and their household contacts. Pediatr Infect Dis J 2007;26(3):238-42

76. Wendelboe AM, Hudgens MG, Poole C, Van Rie A. Estimating the role of casual contact from the community in transmission of Bordetella pertussis to young infants. Emerg Themes Epidemiol 2007;4:15

77. Castagnini LA, Healy CM, Rench MA, et al. Impact of maternal postpartum tetanus and diphtheria toxoids and acellular pertussis immunization on infant pertussis infection. Clin Infect Dis 2012;54(1):78-84

78. Terranella A, Asay GR, Messonnier ML, et al. Pregnancy dose Tdap and postpartum cocooning to prevent infant pertussis: a decision analysis. Pediatrics 2013;131(6): e1748-56

79. Belloni C, De Silvestri A, Tinelli C, et al. Immunogenicity of a three-component acellular pertussis vaccine administered at birth. Pediatrics 2003;111(5 Pt 1):1042-5

80. Wood N, McIntyre P, Marshall H, Roberton D. Acellular pertussis vaccine at birth and one month induces antibody

doi: 10.1586/14760584.2014.946016

responses by two months of age. Pediatr Infect Dis J 2010;29(3):209-15

81. CDC. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in pregnant women and persons who have or anticipate having close contact with an infant aged <12 months – advisory committee on immunization practices (ACIP), 2011. MMWR Morb Mortal Wkly Rep 2011;60(41):1424-6

82. CDC. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (tdap) in pregnant women advisory committee on immunization practices (ACIP), 2012. MMWR Morb Mortal Wkly Rep 2013; 62(7):131-5

83. Gall SA, Myers J, Pichichero M. Maternal immunization with tetanus-diphtheria-pertussis vaccine: effect on maternal and neonatal serum antibody levels. Am J Obstet Gynecol 2011;204(4): 334e331-5

84. Leuridan E, Hens N, Peeters N, et al. Effect of a prepregnancy pertussis booster dose on maternal antibody titers in young infants. Pediatr Infect Dis J 2011;30(7):608-10

85. Munoz FM, Bond NH, Maccato M, et al. Safety and immunogenicity of tetanus diphtheria and acellular pertussis (Tdap) immunization during pregnancy in mothers and infants: a randomized clinical trial. JAMA 2014;311(17):1760-9

86. Warfel JM, Papin JF, Wolf RF, et al. Maternal and Neonatal Vaccination Protects Newborn Baboons From Pertussis Infection. J Infect Dis 2014. [Epub ahead of print]

- **Study demonstrating that both maternal vaccination and neonatal vaccination prevent severe pertussis disease in baboons.**

87. Quinn HE, Snelling TL, Macartney KK, McIntyre PB. Duration of protection after first dose of acellular pertussis vaccine in infants. Pediatrics 2014;133(3):e513-19

88. Nilsson L, Lepp T, von Segebaden K, et al. Pertussis vaccination in infancy lowers the incidence of pertussis disease and the rate of hospitalisation after one and two doses: analyses of 10 years of pertussis surveillance. Vaccine 2012;30(21):3239-47

89. Halasa NB, O'Shea A, Shi JR, et al. Poor immune responses to a birth dose of diphtheria, tetanus, and acellular pertussis vaccine. J Pediatr 2008;153(3):327-32

90. 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(5):655-60.660 e651

91. Hewlett EL, Burns DL, Cotter PA, et al. Pertussis pathogenesis – what we know and what we don't know. J Infect Dis 2014; 209(7):982-5

92. Melvin JA, Scheller EV, Miller JF, Cotter PA. Bordetella pertussis pathogenesis: current and future challenges. Nat Rev Microbiol 2014;12(4):274-88

93. Eby JC, Gray MC, Warfel JM, et al. Quantification of the adenylate cyclase toxin of Bordetella pertussis in vitro and during respiratory infection. Infect Immun 2013; 81(5):1390-8

94. Pawloski LC, Queenan AM, Cassiday PK, et al. Prevalence and molecular characterization of pertactin-deficient Bordetella pertussis in the United States. Clin Vaccine Immunol 2014;21(2):119-25

- **Screened 1300 clinical isolates of B. pertussis to track the rapid emergence of pertactin-deficient strains in the USA.**

95. Queenan AM, Cassiday PK, Evangelista A. Pertactin-negative variants of Bordetella pertussis in the United States. N Engl J Med 2013;368(6):583-4

96. Lam C, Octavia S, Ricafort L, et al. Rapid increase in pertactin-deficient Bordetella pertussis isolates, Australia. Emerg Infect Dis 2014;20(4):626-33

97. Hegerle N, Paris AS, Brun D, et al. Evolution of French Bordetella pertussis and Bordetella parapertussis isolates: increase of Bordetellae not expressing pertactin. Clin Microbiol Infect 2012;18(9):E340-6

98. Otsuka N, Han HJ, Toyoizumi-Ajisaka H, et al. Prevalence and genetic characterization of pertactin-deficient Bordetella pertussis in Japan. PLoS One 2012;7(2):e31985

99. Bart MJ, Harris SR, Advani A, et al. Global population structure and evolution of Bordetella pertussis and their relationship with vaccination. MBio 2014;5:2

- **Used comparative genomics to analyze a worldwide collection of several hundred *B. pertussis* isolates collected between 1920 and 2010. The authors found little evidence of recent geographical clustering, suggesting *B. pertussis* strains are rapidly transmitted between countries.**

100. Bodilis H, Guiso N. Virulence of pertactin-negative Bordetella pertussis isolates from infants, France. Emerg Infect Dis 2013;19(3):471-4

101. van Gent M, van Loo IH, Heuvelman KJ, et al. Studies on Prn variation in the mouse model and comparison with epidemiological data. PLoS One 2011;6(3):e18014