Nonhuman Primate and Human Challenge Models of Pertussis

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Despite pertussis vaccination rates in excess of 95%, pertussis rates in the United States have been rising over the last 30 years, with increasingly larger outbreaks in 2004, 2010, and 2012. The reasons for this resurgence of pertussis are not clearly understood. The recent development of a baboon model of pertussis, along with the future development of a human challenge model of pertussis, has the potential to provide a path forward for answering critical questions about pertussis pathogenesis and host responses and will likely aid in the development of next-generation pertussis vaccines.

Keywords. Animal models; Bacterial vaccines; Pertussis; Pulmonary infections.

Whooping cough is a highly contagious, acute respiratory illness caused by the bacterial pathogen *Bordetella pertussis* [1]. The introduction of pertussis vaccines in the 1940s and nationwide coverage in excess of 95% led to a dramatic decrease in the disease. However, for unexplained reasons, pertussis rates in the United States have been rising over the last 30 years [2]. With >48 000 reported cases in the United States in 2012, pertussis is the most commonly reported vaccine-preventable disease.

A variety of animal models have been used to study the pathogenesis of pertussis, including mice, rabbits, guinea pigs, and newborn piglets [3]. While these models reproduce many aspects of pertussis, none of them adequately reproduce the full spectrum of the disease observed in humans. Experiments on nonhuman primates have brought about important advances in biology and medicine. 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 nonhuman primate species reported that nonhuman primates develop all of the characteristic markers of human pertussis [4–9]. However, these studies were published >50 years ago with limited experimental detail, making it difficult to evaluate those models.

CHIMPANZEES

Two studies were published in the 1930s, in which chimpanzees were directly challenged with *B. pertussis* [7, 9]. In the first study, Rich et al directly inoculated 2 chimpanzees with unfiltered sputum from human cases of pertussis [7]. Both chimpanzees developed a case of pertussis that was described as being indistinguishable from human pertussis. The disease in these animals was characterized by prolonged cough illness accompanied by significant leukocytosis and isolation of *B. pertussis*. A third chimpanzee was inoculated with sputum from one of the 2 infected chimpanzees and also developed typical whooping cough. In a second report, Shibley challenged a single chimpanzee by spraying *B. pertussis* into the face of the animal on 3 successive days [9]. The challenged chimpanzee developed a cough illness, starting on day 9, that was described as paroxysmal by day 14, with 25–30 coughs in succession often followed by emesis. Definite whooping was reported on day 19 after challenge. This animal was euthanized at the peak of infection, at which time the white blood cell (WBC) count was 132 000 cells/μL. At

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autopsy, the findings were described as being typical for findings in human pertussis. Importantly, *B. pertussis* was isolated in the bronchi and bronchioles of this animal, providing evidence that *B. pertussis* colonizes these airways in the course of a typical infection that is uncomplicated by pneumonia. These studies demonstrate that chimpanzees provide an excellent model of pertussis, but ethical considerations preclude their use in pertussis research.

MACAQUES AND CEBUS MONKEYS

Since 1929, 3 studies have reported success in infecting Rhesus monkeys with *B. pertussis*. Sauer and Hambrecht challenged 10 Rhesus monkeys with *B. pertussis* [8]. Three of those monkeys developed mild cases of pertussis characterized by a mild cough of short duration and 2–3-fold rises in WBC counts. In the same study, these researchers challenged 18 *Cebus capucinus* monkeys. Five monkeys developed mild pertussis. Culotta et al challenged 16 Rhesus monkeys and reported mild pertussis signs in 1 animal [4]. In contrast, when these researchers challenged 2 *Erythrocebus patas* monkeys, both animals developed cases of pertussis characterized by a 4–5-fold rise in WBC counts and a cough illness lasting 28 days and 40 days. When Sprunt et al attempted to produce pertussis in *E. patas* monkeys, they met with less success [10]. They challenged 11 *E. patas* monkeys by intratracheal inoculation. All 11 monkeys were colonized, but none of the monkeys exhibited signs of pertussis disease.

Although the 3 studies described above suggest that macaques and Cebus monkeys are not suitable animals for recapitulating human clinical pertussis, 2 studies using *Macaca cyclopis* monkeys challenged that conclusion. Lin inoculated 4 young *M. cyclopis* monkeys with *B. pertussis* and cohoused each inoculated monkey with a naive cage mate [6]. All 8 monkeys developed cases of pertussis characterized by a cough illness lasting 8–24 days and 2–3-fold increases in WBC counts. This study demonstrated experimental transmission of pertussis, as well as protection from subsequent rechallenge. None of the convalescent animals developed pertussis signs upon rechallenge. North et al challenged 10 *M. cyclopis* monkeys with *B. pertussis* [11]. Nine monkeys developed pertussis. The authors stated that paroxysmal coughing was observed in the most severe cases, but the numbers of mild and severe cases were not provided. The range of peak leukocytosis was reported as 15 500–42 600 cells/μL, but the values for individual animals and the preinfection values were not reported. As in the study by Lin, convalescent animals were asymptomatic upon rechallenge. These 2 studies suggest that *M. cyclopis* would possibly provide a useful model of pertussis. If true, it is not clear why *M. cyclopis* monkeys provide a better model than *M. rhesus*, because *M. cyclopis* and *M. rhesus* are closely related species. Unfortunately, *M. cyclopis* monkeys are not readily available as a research model.

As a first step in developing a reliable nonhuman primate model of pertussis, we sought to revisit the suitability of *M. rhesus* monkeys as a model of human clinical pertussis. We challenged 4 young Rhesus monkeys with *B. pertussis* strain D420 [12], a recent clinical isolate that was isolated from a human infant with severe respiratory distress. All 4 monkeys were infected; 2 developed a significant rise in WBC counts (4-fold and 6-fold, respectively), of which only one developed a cough [12]. Two *Macaca fascicularis* monkeys were also challenged with D420. Both monkeys were colonized, but only 1 demonstrated an increased WBC count and developed a mild cough (T. J. M., unpublished data). Carbonetti et al challenged 3 *M. fascicularis* monkeys with strain D420. Only a mild cough of short duration (2–3 days) was observed immediately following challenge, and a mild increase in WBC count was observed in 2 animals (Nicholas Carbonetti, personal communication). Because this recent experience is consistent with previous publications, it is reasonable to conclude that macaques do not provide a useful model of pertussis.

BABOONS

Our results with *M. rhesus* and *M. fascicularis* monkeys led us to explore alternative nonhuman primate models. We inoculated 9 weanling baboons with *B. pertussis* strain D420 [12]. Very high numbers of bacteria were recovered from the nasopharyngeal washes of all 9 baboons from day 2 to day 25 after challenge, and all 9 exhibited very high WBC counts with peak levels 5–10-fold above the preinfection values. All 9 baboons developed severe cough that persisted for >2 weeks. At peak illness, coughing fits were severe, consisting of 5–10 paroxysmal coughs.

As is the case in humans, there is a correlation between the age of the infected animal and the severity of disease. When young infant baboons (5–6 weeks of age) were challenged, severe disease was observed, often resulting in pneumonia (T. J. M., unpublished data). Full-grown adult baboons challenged with the same dose exhibited very mild signs of disease or were asymptomatic (T. J. M., unpublished data). Because 100% of baboons infected with a clinical isolate of *B. pertussis* exhibited the hallmark manifestations of human pertussis, including paroxysmal coughing, mucus production, and leukocytosis, we concluded that young baboons provide an excellent model of pertussis. Since human pathology specimens are only available from *B. pertussis*–infected infants who died of pneumonia, the nonhuman primate model provides an accessible way to study disease mimicking the most common presentation of human pertussis.

It is not known why *B. pertussis* causes only mild infections in macaques but reliably causes severe disease in baboons. Our results suggest that one important factor that contributes to the lack of disease in macaques is the relatively high body

temperature of macaques, compared with that of humans and baboons. *B. pertussis* exhibits a reduced growth rate in vitro at 39°C relative to the rate at 37°C, and adenylate cyclase protein levels are significantly reduced in vitro at 39°C [12]. Both of these temperature-related events may explain the reduced virulence of *B. pertussis* in macaques relative to that in baboons. However, one cannot rule out the possibility that other genetic differences between baboons and macaques contribute to the different susceptibility of these species to *B. pertussis* infection [13, 14].

We subsequently used the baboon model of pertussis to demonstrate transmission of pertussis to uninfected cage mates and to uninfected animals housed in cages 2.1 m away [15]. This was the first demonstration of airborne transmission of *B. pertussis* in a controlled environment in which other modes of transmission could be absolutely ruled out. One of the major early findings from the baboon model is that the host immune response to *B. pertussis* infection results in near-sterilizing immunity [12]. When convalescent baboons were rechallenged, no colonization of the nasopharynx and no rise in circulating WBC counts was observed. The rechallenged animals also displayed robust boosting of serum antibodies beginning on day 5 after challenge, suggesting that T-cell memory may play a substantial role in the sterilizing immunity observed in convalescent baboons. The primary immune response to pertussis was characterized by strong induction of interleukin 6, interleukin 23, and transient interleukin 1β [16]. Consistent with the role of these cytokines in the development of interleukin 17 (IL-17)–producing T cells (Th17 cells), strong expression of IL-17 and several cytokines and chemokines that are orchestrated by Th17 immune responses were detected. The induction of IL-17 and interferon γ–secreting 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 they are due to immunological memory to pertussis. 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 natural *B. pertussis* infection. The demonstration that the immunity conferred by infection in the baboon model is sufficient to protect from subsequent rechallenge demonstrates the usefulness of the baboon model as a tool to fill the gaps in our understanding of infection and vaccine-mediated protection from pertussis.

HUMAN CHALLENGE MODEL OF PERTUSSIS

Human challenge studies with microbial pathogens provide an opportunity to study the natural course of and immune responses to infection. They have contributed significantly to our understanding of bacterial and viral pathogenesis and the immune response to infection. Reviews of previous experience and ethical considerations related to the use of human challenge models for viral and bacterial pathogens are available elsewhere [17–19]. Human challenge models can be used safely and ethically for diseases that are nonfatal and self-limiting in healthy individuals or diseases for which a rescue therapy is available that can be used to quickly bring the participants back to good health [18]. These studies are particularly useful because they can be performed in a controlled setting and because results can be attained with a smaller number of volunteers and more rapidly than studies that require the accumulation of cases as the result of natural exposure in the community. This is particularly relevant for pertussis, which occurs at a relatively low frequency in the population and has an incidence that is sporadic geographically and annually.

Pertussis presents several specific challenges to the design of human challenge studies. Pertussis is highly contagious and transmitted by airborne respiratory droplets. Appropriate containment facilities and inpatient study design are required to prevent transmission to contacts. Antibiotic treatment, with clearance of organism ensured before discharge, and adequate safety follow-up procedures are also required to prevent accidental transmission to subject contacts outside the institution. Antibiotic treatment provides an effective rescue therapy for pertussis when administered early in infection. However, antibiotics have limited or no efficacy when administered late in infection [20]. Although pertussis is generally mild in adults, even mild pertussis in adults has a prolonged duration and is associated with significant discomfort. Severe pertussis is more commonly associated with young children; however, severe pertussis occurs in all age groups. This point of no return with respect to rescue therapy and the potential for severe disease potentially limits pertussis challenge study end points to establishment of infection. Human challenge studies with *B. pertussis* in which subjects are treated as soon as infection is confirmed appear to fall well within the bounds of current ethical and safety standards [18]. Subjects are unlikely to experience unreasonable discomfort, and the infection can be treated and eradicated with confidence. Studies in which subjects who are deliberately infected with *B. pertussis* are allowed to proceed to symptomatic stages of disease may result in infections that cannot be eradicated with complete confidence and may present a risk of significant morbidity to the subject.

INTEGRATION OF HUMAN AND NONHUMAN PRIMATES TO CHARACTERIZE THE PATHOGENESIS OF PERTUSSIS AND THE NATURE OF PROTECTIVE IMMUNITY

The human and nonhuman primate models together provide a way forward to answer questions that have been left unanswered over the last 100 years. The baboon model of pertussis provides the opportunity to follow the full course of natural

B. pertussis infections to better understanding the disease process and the virulence of the organism and to evaluate candidate vaccine antigens and adjuvants in a very relevant animal model. Ultimately, the baboon model may be used to provide proof of concept for vaccines before their study in human clinical trials and could yield information about potential human correlates of protection for candidate vaccines. Once developed, a human challenge model of pertussis could be invaluable for studying early stages of infection, for evaluating the effectiveness of novel antigens and adjuvants in preventing infection, and, depending on the clinical end points, for providing clinical efficacy data for next-generation pertussis vaccines. Together, the baboon and human challenge models of pertussis may facilitate the rational design and testing of next-generation pertussis vaccines in response to the continued resurgence of pertussis.

Notes

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