Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Infant Rhesus Macaques as a Nonhuman Primate Model of *Bordetella Pertussis*

Li Shi (shili.imb@gmail.com)
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
https://orcid.org/0000-0001-9508-7863
Wenwen Jiang
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
Chen Wei
National Institute of Food and Drug Control: China National Institute for Food and Drug Control
Dachao Mou
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
Weilun Zuo
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
Jiangli Liang
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
Xiao Ma
National Institute of Food and Drug Control: China National Institute for Food and Drug Control
Lichan Wang
National Institute of Food and Drug Control: China National Institute for Food and Drug Control
Na Gao
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
Qin Gu
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
peng Luo
National Institute of Food and Drug Control: China National Institute for Food and Drug Control
Yan Ma
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
Jingyan Li
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
Shuyuan Liu
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology
Mingbo Sun
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Medical Biology

Research article

Keywords: Bordetella pertussis, rhesus macaques, infection, transmission

Posted Date: December 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-132288/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background: The prevalent resurgence of pertussis recently creates a vital public health problem worldwide. To understand the pertussis pathogenesis and host response to both pathogen and vaccine, a suitable pertussis animal model, particularly a non-human primate model, is necessary. Recently, a successful non-human primate pertussis model of baboons have been established. However, though the rhesus macaques have been proven to be ideal animal models for several infectious diseases, the infectious model of pertussis has not been established on it. The previous studies on rhesus macaque models of pertussis were performed in 1920s-1930s with limited experimental details. Recent monkey pertussis models failed to be established because the typical clinic syndrome and transmission were not investigated.

Methods: In the present study, infant rhesus macaques were challenged with *Bordetella pertussis* (*B.p)* using the aerosol method to evaluate the feasibility of using it as an animal model of pertussis infection.

Results: Upon aerosol infection, monkeys infected with the recent clinically isolated *B.p* strain 2016-CY-41 developed typical whooping cough, leukocytosis, bacteria-positive nasopharyngeal wash (NPW), and inter-animal transmission. Both humoral and cellular immune responses were induced by *B.pertussis*.

Conclusion: These results demonstrate that a model of pertussis infection was successfully established in infant rhesus macaques, which provides a valuable platform to study pertussis pathogenesis and evaluate vaccine candidates.

Background

Pertussis is an acute respiratory disease caused mostly by the gram-negative bacterium *Bordetella pertussis* (*B.p)*. The basic illness is non-inflammatory in nature and occurs without significant fever. The disease is characterized by non-productive paroxysmal coughs followed by periods of total respiratory normalcy, which makes it different from all other infectious cough illnesses [1]. Severe respiratory failure is complicated by pulmonary hypertension, which may cause death, especially in infants [2]. Pertussis is transmitted directly from human to human, mostly via aerosolized respiratory droplets. As a preventable disease, the incidence of pertussis decreased notably after vaccine immunization. However, it has experienced a resurgence in several countries, even in those countries with nearly universal vaccine coverage in the last 20 years [3–5]. Facing this important public health concern, deeper understanding of the mechanism of pertussis pathogenesis and host response to both pathogen and vaccine is urgently required.

To establish suitable animal models for pertussis infection, several studies were carried out in mouse, rat, rabbit, and piglet models of pertussis [6, 7]. Unfortunately, these models cannot reproduce the full clinical spectrum observed in humans [8, 9]. With respect to studies using non-human primate (NHP) models, a successful baboon model was established [10]. Low-grade fever, paroxysmal coughing, lymphocytosis, a long-lived anti-pertussis toxin (PT) antibody response, protection against subsequent challenge, and

transmission were investigated in this baboon model, which makes the use of the model crucial for studies on the pertussis pathogenesis mechanism as well as for the development of new vaccines and therapeutics [11]. Another NHP, *Rhesus Macaca*, has been evaluated for use as a pertussis model since 1929 but does not replicate the human clinical disease[12–14]. In contrast, 2 studies using *Macaca (M.) cyclopis* investigated the similarity to the human pertussis clinical syndrome[15]. Baboons and macaques are Old World monkeys that were separated approximately 10 million years ago, and rhesus macaques are closely related to *M. cyclopis.* Several disease models have been established using macaques and/or baboons [15]. Moreover, compared to the limited availability, high housing costs, and lack of suitable reagents for baboon use, the use of rhesus macaques offers the advantages of animal availability, low housing costs, and suitable reagent availability [16].

Therefore, we infected rhesus monkeys with *B.p* via aerosol challenge. We investigated clinical symptoms, including leukocytosis, coughing, and nasopharyngeal colonization, analyzed the humoral immune response and cytokine levels, and performed a transmission test to evaluate the suitability of infant rhesus macaques as a potential alternative NHP model for pertussis infection.

Methods

Animals

Infant rhesus macaques used in this study, 5–6 months of age, were obtained from Institute of Medical Biology, Chinese Academy of Medical Sciences (IMBCAMS). The study protocol was approved (DWSP201809002) by the Committee on the Ethics of the IMBCAMS and was conducted in strict accordance with the Guidelines for the Care and Use of Laboratory Animals from the National Research Council of the National Academies and the Guidance for Experimental Animal Welfare and Ethical Treatment by the Ministry of Science and Technology of the People's Republic of China (2006). During the study periods, the monkeys were maintained at Animal Biosafety Level 2, housed individually in cages in a climate-controlled room (temperature of 18-25°C and humidity 30-70%) with a 12 h light/dark cycle, received

food and fruits strictly complying with requirements of animal welfare, and had free access to water. After this experiment, the monkeys were confirmed completely recovered from *B.p* infection, and were put back to Monkey Mountain of IMBCAMS, allowing them to die naturally.

Bacterial Strains and Media

B.p strain 2016-CY-41 used in this study was recently isolated from a patient in China and was obtained from the National Institutes for Food and Drug Control (Beijing, China). The polymorphisms in the pertussis toxin promoter (ptxP), pertussis toxin subunit 1 (ptxA), pertactin (prn), fimbrial (fim)2 and fim3 were assessed by DNA sequencing. The genotype of 2016-CY-41 was ptxP1/ptxA1/prn1/fim2-1/fim3-1. For B.p infection experiments, bacteria were grown on Bordet-Gengou agar (B-G) plates (BG, Hopebio, CHN) containing 20% defibrinated sheep blood (Nanjinglezhen, CHN) for 48 to 72 h at 37°C. Colonies

from fresh B-G plates were resuspended in isotonic saline, diluted to a concentration of 1011 CFU/mL using a turbidimetric method, and used within 2 h of preparation. For the culture of nasopharyngeal wash (NPW) bacteria, Regan-Lowe plates, prepared from Regan-Lowe charcoal agar base with 10% defibrinated sheep blood and 40 μg/mL cephalexin (Oxoid, US), were used.

Infection and Transmission in Rhesus Macaques

Seven healthy male rhesus macaques, aged 5 to 6 months and weighing from 1.2-1.8 kg, were obtained from the IMBCAMS (Animal License No. SCXK (Dian) K2015-0004) and randomly assigned into two groups (Table 1). Group 1, containing 5 macaques were challenged with strain 2016-CY-41 via aerosol exposure using an aerosolization apparatus designed by our lab and produced by Lanfang Honlan Equipment Co (Additional file 1). The apparatus was composed of a rectangular Plexiglas chamber with a removable lid (40 cm × 60 cm × 40 cm), a pump and a medical nebulizer (average atomization rate: ³ 0.15 mL/min, working pressure: 60-150 KPa, normal working condition: 10-40°C). The pump was connected to the inlet side of the nebulizer to deliver a *B.p* suspension for atomization. The outlet side of the nebulizer was connected to two inlet ports of the challenge chamber to deliver atomized B.p to the interior of the chamber. An outlet tube with an air filter was connected to the challenge chamber to remove air. An air sampling port was embedded in the middle of the challenge chamber to monitor the actual concentration of aerosolized *B.p* inside the chamber. Animals were infected via the challenge chamber for 60 min. Within the 60 min period, the air sample was removed from the sampling port every 10 min for the assessment of the concentration of B.p inside the chamber.

At 2 days post-infection (dpi), 1 challenged macaque was cohoused with 1 naive animal in one cage, and the animals were separated after 4 days to investigate transmission. Thus, 2 macaques who were cohoused with 2016-CY-41-challenged animals formed group 2.

Evaluation of Animals and Sample Collection

A schematic of the specimen collection timeline is displayed in Figure 1. Total white blood cell (WBC) counts were measured by blood cell counting. Coughing frequency was monitored by a recording device, which was reviewed, and the number of coughs at four 30-min periods each day (7:00-7:30 a.m., 10:00-10:30 a.m., 2:00-2:30 p.m., and 8:00-8:30 p.m.) was calculated. The average number of coughs per hour for each day was calculated as the mean for all four observation periods for all animals in each group. The NPW was serially diluted in saline and plated on Regan-Lowe plates. The number of CFUs was calculated after 4-5 days of incubation at 37°C. The B.p colonies were identified by examining the colony morphology and hemolysis on Regan-Lowe plates and by polymerase chain reaction (PCR) amplification of IS481, a genomic insertion site that is specific for *B.p*[17].

Detection of Serum Antibodies

Serum was separated, and anti-PT, anti - filamentous hemagglutinin (FHA), anti-PRN and anti- adenylate cyclase toxin (ACT) levels were measured using an enzyme-linked immunosorbent assay (ELISA). A 96-

well micro plate was coated with 3 μg/mL of the antigens PT, FHA, PRN, and ACT and incubated at 4°C overnight. Then, the plates were blocked with 3% (w/v) bovine serum albumin (BSA, Amresco, A0332) in phosphate buffered saline (PBS) at 37°C for 2 h. Diluted serum was added to the microplate and incubated at 37°C for 1 h. After washing, horseradish peroxidase (HRP)-labeled sheep anti-monkey immunoglobulin G (IgG) was added to the microplate, and the plate was incubated at 37°C for 1 h. All of the ELISA plates were developed using tetramethylbenzidine (TMB; Solarbio, PR1200) to generate a colorimetric reaction and terminated with 2 mol/L of H2SO4. For each set of ELISA plates, a WHO international standard pertussis antiserum was used as a reference (NIBSC code: 06/140). For anti-ACT, a blank sample was included in each plate, and optical density (OD) values ≥ 2.1-fold those of the blank sample were set as cutoff values (all the antigens were from the Department of DTP Vaccine and Toxin, National Institute for Food and Drug Control, China). Results were presented as geometric mean concentrations (GMCs) or geometric mean titers (GMTs) and their 95% confidence intervals (CIs).

Measurement of Cytokines

Serum concentrations of interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, IL-12/23p40, IL-13, IL-17A, interferon (INF)-γ, and tumor necrosis factor (TNF)-a were detected by the Luminex technique with a Milliplex NHP Magnetic Bead Panel (Merck Millipore, US) according to the manufacturer's instructions. An unpaired t-test was used to test for differences between the pre-challenge and peak cytokine production post-challenge period (2/4, 6/8, 10/12, 14/16, 21/23, 28/30 dpi, the latter is for the transmission group as Fig. 1 indicated) for each animal due to the highly variable individual starting concentrations between animals and variability of the peak response for each cytokine post-infection.

Statistics

Data were graphed and analyzed using GraphPad Prism version 7.0 (GraphPad Software, Inc.). The data are presented as the mean ± standard errors of the mean or GMCs / or GMTs and their 95% confidence intervals (CIs). Unpaired student's t-test was utilized to assess statistical significance.

Results

Clinical Signs in Infant Rhesus Macaques after B.p Infection

The concentration of bacteria in the challenge chamber reached and was maintained at 104–105 CFU/mL. In challenged group, all 5 animals developed classic symptoms of clinical pertussis. The number of WBCs was significantly increased 2-5-fold, beginning at 6 dpi, reached the highest level at 14 dpi, and returned to baseline at 28 dpi (Fig. 2A). The number of bacterial colonies from the nasopharyngeal wash (NPW) increased from 2 dpi and reached the highest level, 6.2 × 106 CFU/mL, at 10 dpi, and then, the number of colonies was gradually decreased until approximately 45 dpi (Fig. 2B). In addition, all animals developed severe coughs that persisted for over 4 weeks. In the early stage after challenge, animals developed a mild cough. At 10 dpi, the cough seemed to worsen, especially at night (Fig. 2C). At peak illness, the cough became violent, lasting 10–20 seconds (Additional file 2). However,

the rectal temperature was not significantly different from that in the pre-challenge period and was maintained between 37.2°C and 39.9°C (Additional file 3).

Antibody Response

The antibodies against PT, FHA, PRN, and ACT were induced in all 5 macaques in challenged group. The seroconversion rate reached 100% at day 14 for anti-PT and anti-FHA. As for anti-PRN, the seroconversion rate reached 100% at day 35 with a little delay, but it reached 60% at day 14, and remained at 80% from day 28 during the investigating period. The anti-PT, anti-FHA and anti-PRN antibodies significantly increased from 14 days and reached to approximately 200-fold, 22-fold, and 11-fold on day 35, respectively. And it remained at a high level with GMCs of 598.9 IU/mL (95% CI, 559.7-640.8), 112.0 IU/mL (95% CI, 84.11–149.2) and 9.8 IU/mL (2.08–45.88) on day 45, respectively (Fig. 3A-C). The anti-ACT was significantly elevated from day 14 and remained stable with GMTs of 9.79 (95% CI,7.84–12.23), 10.82(95% CI,8.91–13.14), and 10.68(95% CI,8.96–12.74), respectively, on days 28, 35, and 45 (Fig. 3C).

Transmission

Four days after separation from the 2016-CY-41-challenged macaques, both macaques in group 2 became infected, as demonstrated by the prominent leukocytosis, with a peak level between 2- and 4-fold greater than the pre-infection level (Fig. 4A), and B.p was recovered from the NPW, with the highest numbers reaching 5.4 × 106 and 7.0 × 106 CFU/mL (Fig. 4B). More importantly, both animals were also developed severe coughs (Fig. 4C). Antibody responses to B.p were also observed and were similar to the trend seen following primary infection in group 1(Fig. 4D-G).

Cytokine Levels

The expression of the proinflammatory cytokines IL-6, IL-1β and TNF-α was significantly upregulated in all 7 macaques infected with strain 2016-CY-41. In addition, the increased IL-12/23p40 and IL-10 responses suggested that there was increased T cell activation and regulation in macaques after infection (Fig. 5).

Discussion

Pertussis is a vaccine-preventable childhood disease; however, there has been a resurgence in cases in recent years, including in counties with good vaccine immunization rates. A deep understanding of the immunology and epidemiology of this pathogen through suitable experimental models, particularly nonhuman primate models, is important. Contrary to previous rhesus monkey challenge studies, in which most animals infected with pertussis did not develop obvious clinical manifestations of human pertussis

[10, 12, 15], we investigated the pertussis clinical spectrum in a rhesus macaque model in the present study. First, the characteristic whooping cough of pertussis syndrome, which has been investigated in only the baboon model [10], was investigated. All 5 challenged macaques and 2 macaques cohoused with the challenged macaques developed severe coughs that persisted for over 4 weeks. The coughing appeared on day 2, peaked at day 15, and decreased gradually thereafter. At peak illness, the coughing became severe, lasting 10–20 seconds. In addition, the number of colonies in the NPW peaked at day 10 after challenge, reaching 6.2 × 106 CFU/mL, and returned to baseline levels after 35 days.

One of the most important factors to consider when studying the above systems is the method of challenge. To date, nasal challenge, endotracheal intubation, in vivo injection, and aerosol challenge have been investigated in mouse models of pertussis [18, 19]. Compared to other challenge methods, aerosol challenge can accurately simulate natural infections and reduce animal stress [20]. We previously infected rhesus monkeys with strain 18323 at a concentration of 5 × 108 CFU/mL with a 2 mL volume through nasal challenge but did not investigate clinical signs (data not shown). Using an aerosol apparatus, we challenged mice with different concentrations at different aerosol challenge times and successfully established a B.p infection in a mouse model [21]. In the present study, using the aerosol apparatus, we investigated the whole spectrum of pertussis infection. Compared to other methods of experimental infection, aerosol challenge has great value due to the accurate simulation of natural infections, the superior reproducibility and predictable distribution of infection and pathology [22, 23].

Another critical factor to consider when establishing an animal model is the age of the animals. A previous study using an enterovirus type 71 (EV71) rhesus macaques model showed that, of the challenged animals, clinical spectrum similar to that of humans was observed in only young animals [24]. Previous studies involving rhesus monkeys challenged with pertussis investigated the fold-change in WBC counts and cough but did not evaluate clinical manifestations in adult animals [12, 14]. In contrast, the whooping cough, dynamic changes in leukocytosis and the antibody response were observed in the Taiwanese monkey (M. cyclopis) challenged with B.p strain 18323 [25]. Although weight was recorded for these monkeys (weighed from 550 to 1875), age was not available. Moreover, studies have demonstrated that young baboons showed severe disease signs, whereas adult baboons showed mild signs [10]. In our study, rhesus macaques aged 5 to 6 months were selected, and typical whooping cough, leukocytosis, bacteria-positive NPW, and transmission between animals were observed, which is similar to the results obtained in the baboon model. Therefore, we deduced that the challenge route, as well as the age of the animals, may influence the B.p infection.

The strain of challenge bacteria is another factor that should be noted. Strain 18323 was used successfully as an infection strain in pertussis infection models in Macaca cyclopsis in the 1960s; however, it could not induce overt signs of disease in rhesus macaca [26]. Recent analysis of the global population structure of B.p indicated that strain 18323 (genotype ptxP4/ptxA5/prn6/fim2-2/fim3-1) belongs to the branch containing a small number of strains that has a long distance away from the major prevalent branch, indicating that the strain 18323 were separated from the prevalent strains branch approximately 2,000 years ago [27]. Thus, on contrary to previous macaque animal study, we selected a

recent clinically isolated strain in China, 2016-CY-41 (genotype ptxP1/ptxA1/prn1/fim2-1/fim3-1), carrying the prevelant common stainst, and investigated the typical pertussis symptoms in the infected macaques.

Compared with the baboon model infected using strain D420 (genotype ptxP3/fim3-2), the symptom and disease progression peak in the B.p strain 2016-CY-41-infected macaque model was delayed [10]. PtxP3, one of the major components of D420, have increased in many European counties, the US, and Australia in the past 25 to 30 years instead of ptxp1, but not in China, where ptxP1 has remained predominant [10, 28, 29]. The SNP in ptxP3 lies in a biding site for transcription regular BvgA, and it may result in a strong promoter and increase the level of transcription of the associated pertussis toxin [30]. Strains harboring the ptxP3 allele ware more virulent than ptxP1 strains in a mouse model infection, and it may also be associated with severe disease in human beings [30–32]. Thus, we hypothesize that genomic diversity of B.p may affect the pertussis infection model. Further whole-genome sequencing and virulence mechanism and pertussis epidemiology analyses should be performed in the future.

One of the considerations of an unsuccessful rhesus model of pertussis infection is high body temperature. The results of a temperature culture test in vitro showed that adenylate cyclase protein levels were significantly reduced in cells grown at 39 °C compared to those in cells grown at 37 °C, supporting the hypothesis that high temperature (39 °C) may result in the loss of expression of ACT, in turn, resulting in no B.p infection[10]. However, pathogenic microorganisms cultured in vitro may exhibit virulence factor loss due to the lack of the host selective pressures. During nonrandom culturing, B.p can undergo spontaneous phase variation, involving multistep disappearance of virulence factors in the following order: ACT, PT and FHA [33]. The results of experiment in vitro suggested that the reduced expression of ACT was caused by the elevated normal body temperature of rhesus macaques [10, 34]. In the present in vivo study, we observed the anti-ACT antibody level increased 20-fold, which was similar to that observed in the baboon model [35]. In addition, the rectal temperature was between 37.2 °C and 39.9 °C and did not exhibit significant changes. Thus, we deduced that body temperature may not be the only reason for the failed rhesus monkey pertussis infection models.

Conclusion

An infant rhesus macaque model of pertussis was established via aerosol challenge to provide a valuable alternative platform to study pertussis pathogenesis and evaluate vaccine candidates.

Abbreviations

B.p Bordetella pertussis

NHP Non-human primate

ptxP pertussis toxin promoter

ptxA pertussis toxin subunit

prn pertactin

NPW nasopharyngeal wash

dpi days post-infection

WBC white blood cell

FHA filamentous hemagglutinin

ACT adenylate cyclase toxin

Declarations

Competing interests

The author(s) declare that they have no competing interests.

Authors' Contributions

SL and MB conceived and designed the study. WW, WC, and DC performed the study. WL, JL, MX, LC, GN, GQ, and LP. provided the necessary research reagents, animal and technical expertise. MY, JY, and SY analyzed the data. SL, MB, and WW wrote the manuscript. All authors have read and approved the final manuscript.

Funding

This study was funded by the National Health and Family Planning Commission of China (2015ZX09101031), Yunnan Provincial Science and Technology Department (2016GA004, 2016ZF003 and 2019HC006), and CAMS Initiative for Innovative Medicine (2016-I2M-1-019). These funding agencies were not involved in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We are grateful to the research staff at the Department of Nonhuman Primate Research Center of the Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College for their contribution to this research, and to the Department of Diphtheria, Tetanus, and Pertussis Vaccine and Toxins of the National Institutes for Food and Drug Control for assisting in *B.p* strain 2016-CY-41.

References

1. Cherry JD. The prevention of severe pertussis and pertussis deaths in young infants. Expert Rev Vaccines. 2019;18(3):205–8.

2. Paddock CD, Sanden GN, Cherry JD, Gal AA, Langston C, Tatti KM, Wu KH, Goldsmith CS, Greer PW, Montague JL, et al. Pathology and pathogenesis of fatal Bordetella pertussis infection in infants. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2008;47(3):328–38.
3. Barkoff AM, Mertsola J, Pierard D, Dalby T, Hoegh SV, Guillot S, Stefanelli P, van Gent M, Berbers G, Vestrheim D, et al: **Pertactin-deficient Bordetella pertussis isolates**: **evidence of increased circulation in Europe**, 1998 **to 2015**. *Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2019, **24**(7).
4. Pawloski LC, Queenan AM, Cassiday PK, Lynch AS, Harrison MJ, Shang W, Williams MM, Bowden KE, Burgos-Rivera B, Qin X, et al. Prevalence and molecular characterization of pertactin-deficient Bordetella pertussis in the United States. Clin Vaccine Immunol. 2014;21(2):119–25.
5. Weigand MR, Pawloski LC, Peng Y, Ju H, Burroughs M, Cassiday PK, Davis JK, DuVall M, Johnson T, Juieng P, et al. Screening and Genomic Characterization of Filamentous Hemagglutinin-Deficient Bordetella pertussis. Infect Immun. 2018;86(4):e00869-00817.
6. Van Der Ark AA, Hozbor DF, Boog CJ, Metz B, Van den Dobbelsteen G, van Els CA. Resurgence of pertussis calls for re-evaluation of pertussis animal models. Expert Rev Vaccines. 2012;11(9):1121–37.
7. Elahi S, Holmstrom J, Gerdts V. The benefits of using diverse animal models for studying pertussis. Trends Microbiol. 2007;15(10):462–8.
8. Vandebriel RJ, Hellwig SM, Vermeulen JP, Hoekman JH, Dormans JA, Roholl PJ, Mooi FR. **Association of** *Bordetella pertussis* **with host immune cells in the mouse lung**. Microb Pathog. 2003;35(1):19–29.
9. Safarchi A, Octavia S, Luu LD, Tay CY, Sintchenko V, Wood N, Marshall H, McIntyre P, Lan R. **Better colonisation of newly emerged** *Bordetella pertussis* **in the co-infection mouse model study**. Vaccine. 2016;34(34):3967–71.
10. Warfel JM, Beren J, Kelly VK, Lee G, Merkel TJ. Nonhuman primate model of pertussis. Infect Immun. 2012;80(4):1530–6.
11. Melvin JA, Scheller EV, Miller JF, Cotter PA. *Bordetella pertussis* **pathogenesis: current and future challenges**. Nat Rev Microbiol. 2014;12(4):274–88.
12. North EA, Keogh EV. GA. CR: Experimental pertussis in the monkey. Aust J Exp Biol Med Sci. 1940;18:125–30.
13. Rich ARLP, Brown JH, Bliss EA, Holt LE. : **Experiments upon the cause of whooping cough**. Science. 1932;76:330–1.
14. Sauer LW. H. L: Experimental whooping cough. Am J Dis Child. 1929;37:732–44.
15. Hewlett EL, Burns DL, Cotter PA, Harvill ET, Merkel TJ, Quinn CP, Stibitz ES. Pertussis pathogenesis–what we know and what we don't know. The Journal of infectious diseases. 2014;209(7):982–5.
16. Vinson A, Prongay K, Ferguson B. The value of extended pedigrees for next-generation analysis of complex disease in the rhesus macaque. Ilar J. 2013;54(2):91–105.

17. van der Zee A, Schellekens JF, Mooi FR. Laboratory Diagnosis of Pertussis. Clin Microbiol Rev. 2015;28(4):1005–26.
18. Kwon HJ, Han SB, Kim BR, Kang KR, Huh DH, Choi GS, Ahn DH, Kang JH. Assessment of safety and efficacy against Bordetella pertussis of a new tetanus-reduced dose diphtheria-acellular pertussis vaccine in a murine model. BMC Infect Dis. 2017;17(1):247–53.
19. Wilk MM, Misiak A, McManus RM, Allen AC, Lynch MA, Mills KHG: **Lung CD4 Tissue-Resident Memory T Cells Mediate Adaptive Immunity Induced by Previous Infection of Mice with Bordetella pertussis**. *Journal of immunology (Baltimore, Md*: 1950) 2017, **199**(1):233–243.
20. Sato Y, Izumiya K, Sato H, Cowell JL, Manclark CR. **Aerosol infection of mice with** *Bordetella pertussis*. Infect Immun. 1980;29(1):261–6.
21. Mou D, Liang J, Gao N, Gu Q, Zhang M, Dai Y, Ji Q, Sun M, Yang H. **Aerosol challenge model in murine by** *Bordetella pertussis*. J Postgrad Med. 2017;30(8):808–12.
22. Izumiya K. **Aerosol infection of micewith** *Bordetella pertussis*. Infect Immun. 1980;29(1):261–6.
23. Halperin SA, Heifetz SA, Kasina A. **Experimental respiratory infection with** *Bordetella pertussis* **in mice: comparison of two methods**. Clinical investigative medicine Medecine clinique et experimentale. 1988;11(4):297–303.
24. Zhao T, Zhang Z, Zhang Y, Feng M, Fan S, Wang L, Liu L, Wang X, Wang Q, Zhang X, et al. Dynamic Interaction of Enterovirus 71 and Dendritic Cells in Infected Neonatal Rhesus Macaques. Front Cell Infect Microbiol. 2017;7:1–13.
25. Huang CC, Chen PM, Kuo JK, Wen HC, Lin ST, Lin HS, Lin YC. Experimental whooping cough. N Engl J Med. 1962;266(3):105–11.
26. Huang CC, Chen PM, Kuo JK, Chiu WH, Lin ST, Lin HS, Lin YC. Experimental Whooping cough. N Engl J Med. 1962;266:105–11.
27. Bart MJ, Harris SR, Advani A, Arakawa Y, Bottero D, Bouchez V, Cassiday PK, Chiang C-S, Dalby T, Fry NK, et al. Global Population Structure and Evolution of Bordetella pertussis and Their Relationship with Vaccination. mBio. 2014;5(2):e01074-01014.
28. Li L, Deng J, Ma X, Zhou K, Meng Q, Yuan L, Shi W, Wang Q, Li Y, Yao K: **High Prevalence of Macrolide-Resistant Bordetella pertussis and ptxP1 Genotype**, **Mainland China**, 2014–**2016**. *Emerg Infect Dis* 2019, **25**(12):2205–2214.
29. Xu Y, Zhang L, Tan Y, Wang L, Zhang S, Wang J. Genetic diversity and population dynamics of Bordetella pertussis in China between 1950–2007. Vaccine. 2015;33(46):6327–31.
30. Mooi FR, van Loo IH, van Gent M, He Q, Bart MJ, Heuvelman KJ, de Greeff SC, Diavatopoulos D, Teunis P, Nagelkerke N, et al. Bordetella pertussis strains with increased toxin production associated with pertussis resurgence. Emerg Infect Dis. 2009;15(8):1206–13.
31. Clarke M, McIntyre PB, Blyth CC, Wood N, Octavia S, Sintchenko V, Giles L, Quinn H, Hill V, Hanly G, et al. The relationship between Bordetella pertussis genotype and clinical severity in Australian children with pertussis. J Infect. 2016;72(2):171–8.

32. King AJ, Lee Svd, Mohangoo A, Gent, Mv. Ark Avd, Waterbeemd Bvd: **Genome-Wide Gene Expression Analysis of Bordetella pertussis Isolates Associated with a Resurgence in Pertussis: Elucidation of Factors Involved in the Increased Fitness of Epidemic Strains**. *PloS one* 2013.
33. Goldman S, Hanski E, Fish F. **Spontaneous phase variation in** *Bordetella pertussis* **is a multistep non-random process**. EMBO J. 1984;3(6):1353–6.
34. Watanabe M, Connelly B, Weiss AA. Characterization of serological responses to pertussis. Clinical vaccine immunology: CVI. 2006;13(3):341–8.
35. Eby JC, Gray MC, Warfel JM, Paddock CD, Jones TF, Day SR, Bowden J, Poulter MD, Donato GM, Merkel TJ, et al. **Quantification of the adenylate cyclase toxin of** *Bordetella pertussis in vitro* **and during respiratory infection**. Infect Immun. 2013;81(5):1390–8.

Tables

Table 1: Experimental grouping for *Bordetella pertussis* infection in rhesus macaques

Group	Monkey ID	Sex	Age(month)	Weight(kg)	*B.p* Strain	Infection route
Group 1	18089	M	6	1.5	2016-CY-41	Aerosol challenge
	18105	M	6	1.8	2016-CY-41	Aerosol challenge
	18043	M	5	1.1	2016-CY-41	Aerosol challenge
	18053	M	5	1.2	2016-CY-41	Aerosol challenge
	18093	M	6	1.5	2016-CY-41	Aerosol challenge
Group 2	18073	M	6	1.5	2016-CY-41	Transmission
	18107	M	5	1.2	2016-CY-41	Transmission

Figures

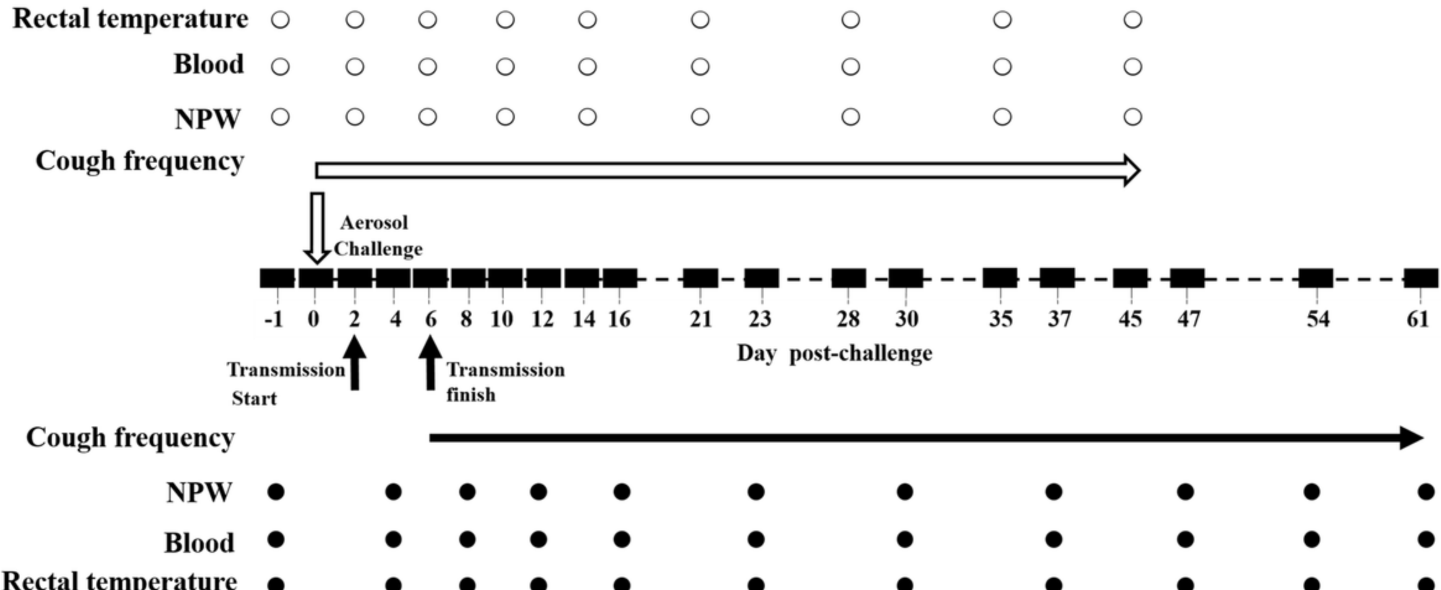
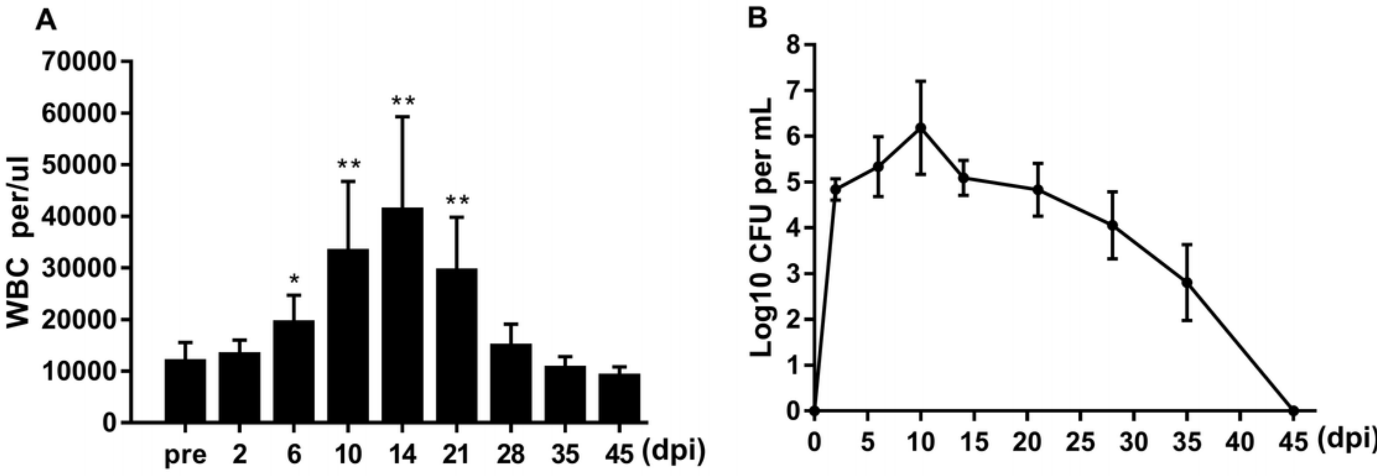


Figure 1

Timeline of B.p aerosol challenge and sample collection from rhesus macaques. Infant rhesus macaques were infected with B.p by aerosol challenge on day 0 (hollow arrow). A naive animal and a challenged animal were cohoused in one cage at 2 dpi for the transmission of the infection (solid arrow). Balls indicate detection time points (hollow for aerosol-challenged animals; solid for transmission animals). Coughing frequency was monitored every day.



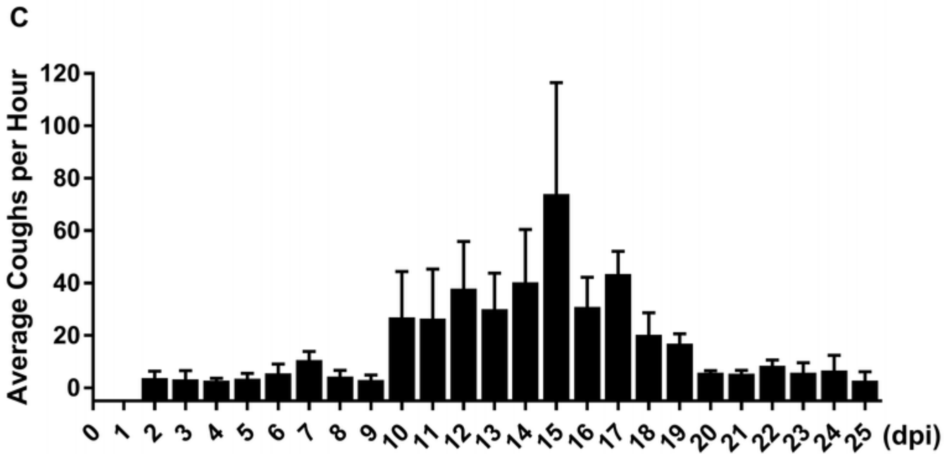


Figure 2

Leukocytosis, B.p colonization, and coughing in B.p-infected rhesus macaques. (A) Dynamic profiles of the number of WBCs per μl of peripheral blood in B.p infected animals. (B) The CFUs per 50 μl of NPW in B.p-infected animals was recorded. (C) The number of coughs per day was recorded for the B.p s-infected group. ND, no data. **, P < 0.01; **, P <0.005. Bars represent mean ± s.d. (n=5).

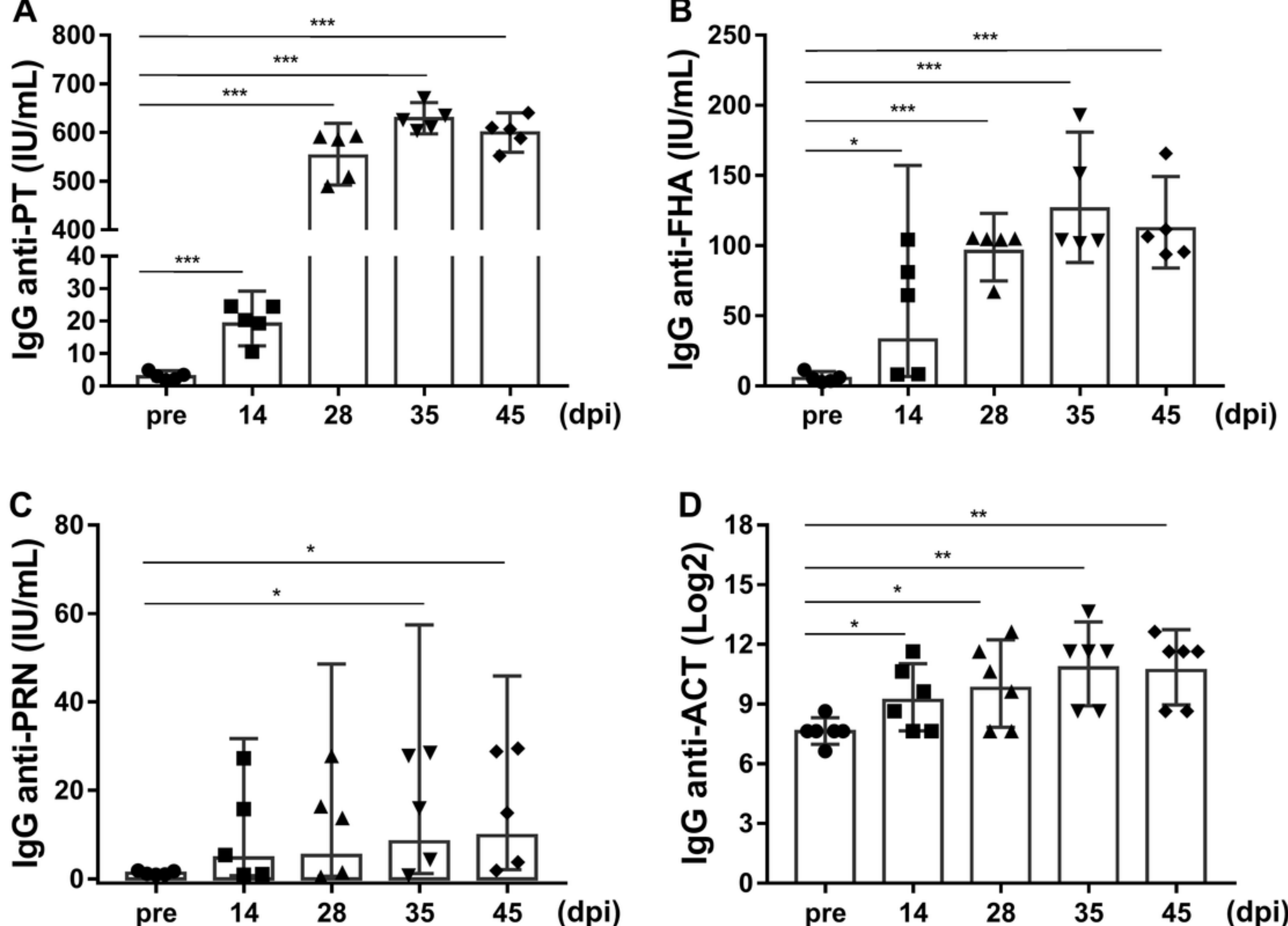


Figure 3

Serological responses to PT, FHA, PRN and ACT in B.p-infected rhesus macaques. Results were presented as geometric mean concentrations (GMCs) or geometric mean titer (GMT) and their 95% confidence interval (CI). (A) Anti-PT; (B) anti-FHA; (C) anti-PRN; (D) anti-ACT. *, P<0.05, **, P<0.01; ***, P<0.001 vs. pre-challenge; Student’s t-test. Bars represent geometric mean with 95% CI. (n=5).

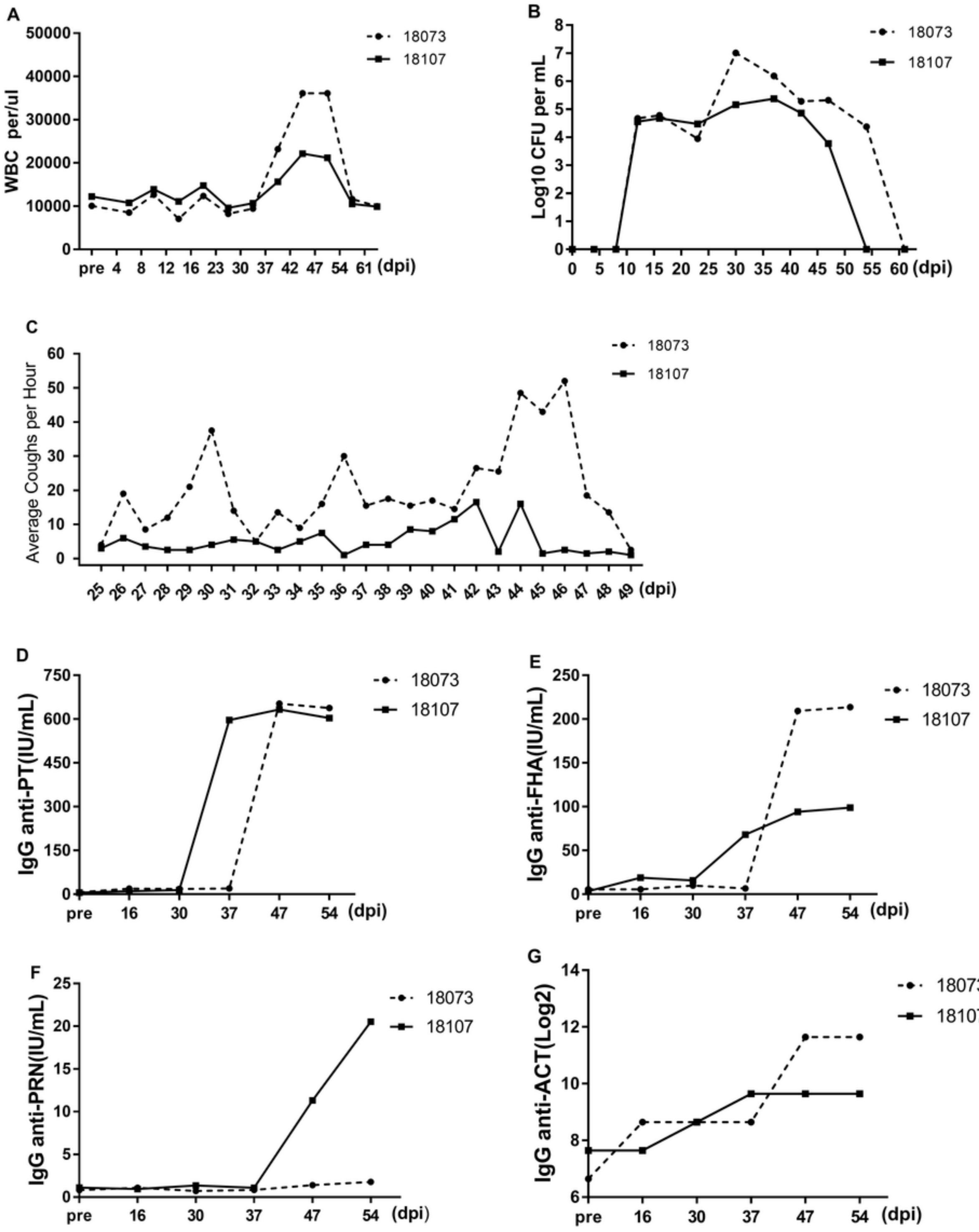


Figure 4

Leukocytosis, B.p colonization, and coughing in group 2 of B.p -transmitted rhesus macaques. At 2 days after challenge, 1 naive animal and 1 challenged macaque were placed in one cage for infection transmission and separated after 4 days. (A) Dynamic profiles of the number of WBCs per μl of peripheral blood. (B) The number of CFUs per 50 μl of NPW was recorded. (C) The number of coughs per day was recorded. (D-G) Antibody responses to the 4 B.p antigens (PT, FHA, PRN and ACT).

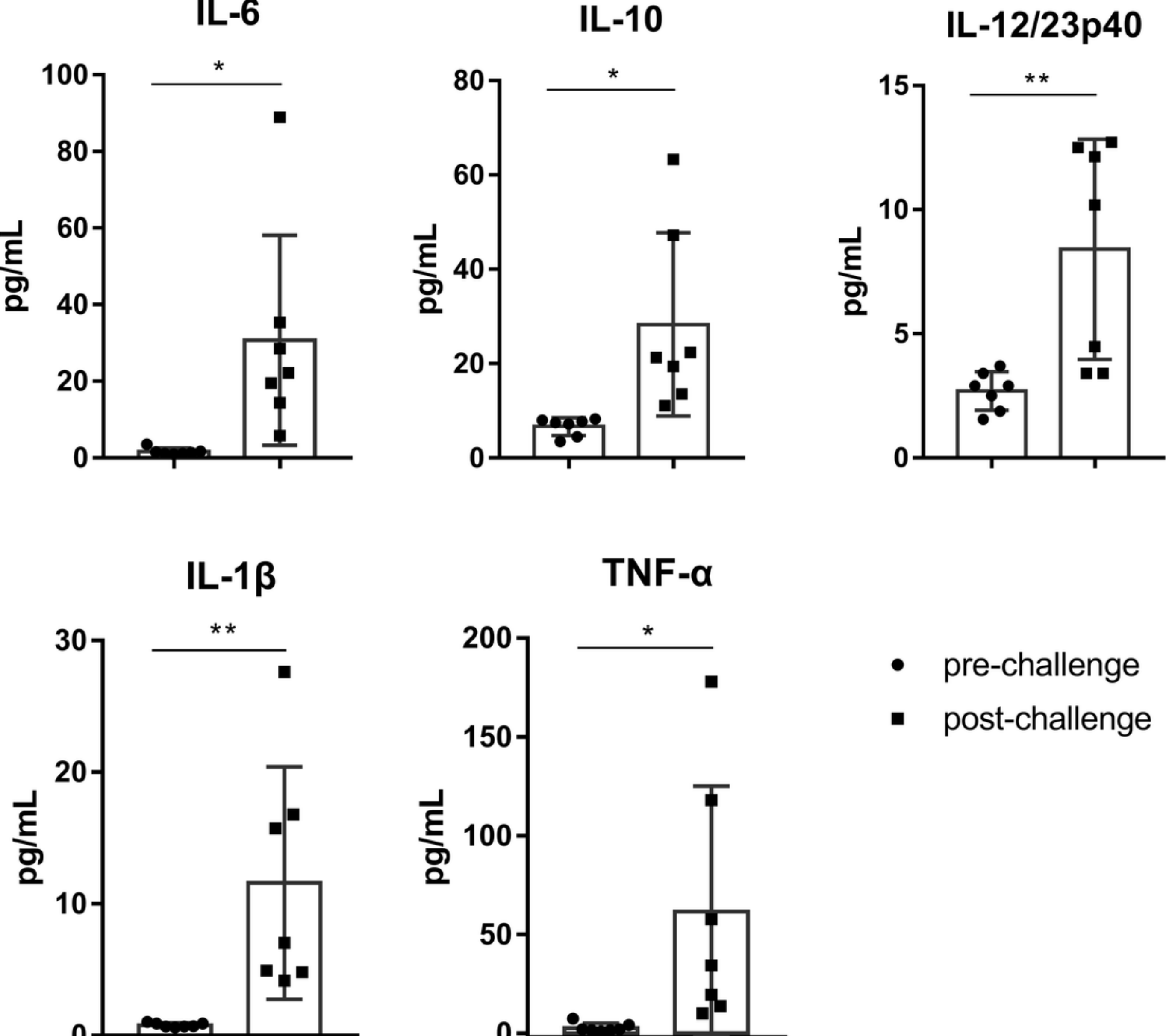


Figure 5

Detection of cytokines in the blood after B.pinfection. Serum was collected from all 7 animals 1 day before challenge and 2, 6, 10, 14, 21, and 28 days post-challenge for group 1 with 2016-CY-41changed, and 4, 8, 12, 16, 23, and 30 days post-challenge for group 2 of transmission macaques cohoused with challenged animals. An unpaired t-test was used to test for differences between pre-infection sera cytokine levels and peak sera cytokine levels obtained at 2-30 dpi with B.p strain 2016-CY-41, since individual starting concentrations were highly variable between animals, as was the day of the peak sera cytokine response. **P < 0.01, *P < 0.05 vs. pre-challenge; Student's t-test. Bars represent mean ± s.d. (n=7).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ARRIVEChecklistsBMCinfectiousdiseases.docx
- Additionalfile1.docx
- Additionalfile2.mp3
- Additionalfile3.docx