HHS Public Access

Author manuscript

Vaccine. Author manuscript; available in PMC 2018 January 25.

Published in final edited form as:

Vaccine. 2015 December 16; 33(51): 7217–7224. doi:10.1016/j.vaccine.2015.10.124.

Efficacy of parainfluenza virus 5 (PIV5)-based tuberculosis vaccines in mice

Zhenhai Chen[*,$,1], **Tuhina Gupta**[*,1], **Pei Xu**[%,1], **Shannon Phan**[1], **Adrian Pickar**[1], **Wilson Yau**[2], **Russell K. Karls**[1], **Frederick D. Quinn**[1], **Kaori Sakamoto**[2], and **Biao He**[1,Π]

[1]Department of Infectious Diseases, University of Georgia College of Veterinary Medicine, Athens, Georgia, USA

[2]Department of Pathology, University of Georgia College of Veterinary Medicine, Athens, Georgia, USA

Abstract

Mycobacterium tuberculosis, the etiological agent of tuberculosis (TB), is an important human pathogen. Bacillus Calmette–Guérin (BCG), a live, attenuated variant of *Mycobacterium bovis*, is currently the only available TB vaccine despite its low efficacy against the infectious pulmonary form of the disease in adults. Thus, a more-effective TB vaccine is needed. Parainfluenza virus 5 (PIV5), a paramyxovirus, has several characteristics that make it an attractive vaccine vector. It is safe, inexpensive to produce, and has been previously shown to be efficacious as the backbone of vaccines for influenza, rabies, and respiratory syncytial virus. In this work, recombinant PIV5 expressing *M. tuberculosis* antigens 85A (PIV5-85A) and 85B (PIV5-85B) have been generated and their immunogenicity and protective efficacy evaluated in a mouse aerosol infection model. In a long-term protection study, a single dose of PIV5-85A was found to be most effective in reducing *M. tuberculosis* colony forming units (CFU) in lungs when compared to unvaccinated, whereas the BCG vaccinated animals had similar numbers of CFUs to unvaccinated animals. BCG-prime followed by a PIV5-85A or PIV5-85B boost produced better outcomes highlighted by close to three-log units lower lung CFUs compared to PBS. The results indicate that PIV5-based *M. tuberculosis* vaccines are promising candidates for further development.

[Π]Corresponding author: Mailing address: Department of Infectious Diseases, University of Georgia College of Veterinary Medicine, 501 D. W. Brooks Dr., Athens, GA 30602. Phone: (706) 542-2855. Fax: (706) 542-5771. bhe@uga.edu.

[*]Authors contributing equally.

[$]Current address: Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, Kansas, United States of America.

[%]Current address: Department of Molecular Genetics and Cell Biology, University of Chicago, Illinois, United States of America.

Conflict of Interest

B.H. is the inventor of a patent application on using parainfluenza virus 5 as a vector for vaccine development that is being pursued by the University of Georgia Research Foundation.

INTRODUCTION

Mycobacterium tuberculosis, the etiological agent of tuberculosis (TB), is an intracellular bacterial pathogen that claimed over one million lives annually and potentially has infected one-third of the world's population. The bacillus Calmette–Guérin (BCG) vaccine, which is an attenuated variant of *M. bovis* developed nearly a century ago, is used to vaccinate newborns against TB. While BCG immunization seems helpful in infants and young children, reducing the incidence of severe, disseminated forms of the disease, it offers variable levels of protection against the more prevalent pulmonary forms of TB in adults [1, 2]. BCG vaccination has made no evident impact on the global TB epidemic. The estimated number of new cases of TB and the per capita incidence worldwide continues to rise each year [3, 4].

The best hope for bringing the global epidemic of TB under control is the development of a new, effective TB vaccine [5–8]. *M. tuberculosis* expresses and secretes three closely related mycolyl transferases also known as the antigen 85 (Ag85) protein complex (Ag85A, 85B and 85C). Both Ag85A and 85B have been shown to be among the most potent antigen species yet identified and are major targets of human T cell responses to *M. tuberculosis* and are leading components in vaccine candidates [9–12]. The various vaccine constructs expressing these antigens have been shown to afford levels of protection in mice, guinea pigs, and monkeys when administered by parenteral and aerosol routes[13, 14]. One particular vaccine candidate, a live recombinant modified vaccinia virus Ankara (MVA) expressing Ag85A (MVA85A) provided significant protection when administered following BCG vaccination in small vertebrate models [15]. It also enhanced vaccine and naturally-primed responses in humans. Unfortunately, the recently-completed Phase 2 human clinical trial measuring protection in infants intradermally BCG prime-vaccinated followed by intradermal boost inoculations with MVA85A did not demonstrate sufficient efficacy [16]. In our study, the intranasal vaccination was examined, with and without an intradermal BCG prime inoculation. The hypothesis is that intranasal immunization is superior against a pulmonary pathogen as it capitalizes on the location and capacity for generating mucosal immunity in the lungs by the parainfluenza virus 5 (PIV5)-based vaccine candidates.

PIV5 is a member of the *Rubulavirus* genus of the family *Paramyxoviridae*, which includes mumps virus and human parainfluenza virus type 2 [17]. It is believed that PIV5 may contribute to kennel cough in dogs [18–22]. Dogs are vaccinated intranasally and often sneeze during the vaccination procedure, exposing veterinary workers and owners. Dogs also shed viruses for five days on average after vaccination [23]. Kennel cough vaccines containing live PIV5 have been used in dogs for several decades without any safety issues for animals and humans.

PIV5 as a prototypical paramyxovirus has been well studied. This virus is a good vector for vaccine development, because it does not have a DNA phase in its life cycle, and thus, the possible unintended consequences of genetic modifications of host cell DNA are avoided. The genome structure of PIV5 is stable. A recombinant PIV5 expressing GFP was maintained for more than 10 generations (the duration of the experiment) without loss of the GFP gene [24]. PIV5 infects a large range of cell types including primary human cells and

various established human cell lines [17, 25, 26]. PIV5 can be grown in Vero cells, a WHO-approved cell line for vaccine production. The virus infects a large number of mammals without being associated with any diseases, except kennel cough in dogs [24–28]. PIV5 has been successfully used to develop vaccines for influenza viruses, respiratory syncytial virus, and rabies virus in animals [27–31]. In a recent study, dogs with pre-existing anti-PIV5 antibodies generated equally robust immune responses as dogs without these antibodies after immunization with PIV5 expressing HA of influenza virus [32], indicating that pre-existing anti-PIV5 antibody did not affect the immunogenicity of a PIV5-based vaccine.

Viral vectors have been widely explored for vaccine development. Adenovirus (AdV)- and vaccinia virus (VV)-based vaccines have been the most extensively studied. While AdV expressing HA is efficacious, protection afforded by AdV expressing NP (AdV-NP) of influenza A virus has been less robust. The best result reported to date using single inoculation of replication-deficient AdV-NP of 10^{10} virus particles was 80% protection of mice from lethal H1N1, but the mice lost close to 30% body weight [33]. There is no report of success of a single inoculation of AdV-NP against H5N1 challenge in mice.

Recent failure of an AdV-based HIV vaccine candidate in clinical trial has also dampened enthusiasm for this viral vector [34]. VV (MVA, modified vaccinia virus-Ankara strain, which has a deletion in the late genes) expressing HA works well, however VV expressing NP did not provide any immunity against lethal influenza virus challenge [35]. PIV5 expressing NP protects 100% of mice against lethal influenza virus H1N1 challenge in mice, demonstrating that PIV5-NP is superior to AdV- and MVA-expressing NP. In this work, recombinant PIV5 expressing *M. tuberculosis* antigen 85A or 85B was generated and protective efficacy tested using the *M. tuberculosis* murine aerosol infection model.

MATERIALS AND METHODS

Cells and Viruses

BHK21 and BSR-T7 cells were maintained in Dulbecco's modified Eagle medium (DMEM) with 10% tryptose phosphate broth (TPB), 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin. MDBK cells were maintained in the same medium without TPB. All cells were incubated at 37°C and 5% CO_2. Plaque assays were performed using BHK21 cells. For virus infection, monolayers were washed with phosphate-buffered saline (PBS) and then inoculated with virus in DMEM plus 1% bovine serum albumin. The infected cells were incubated with DMEM containing 2% FBS at 37°C with 5% CO_2. PIV5 was grown in the MDBK cells [24].

Generation of PIV5 expressing *M. tuberculosis* antigen 85A or 85B

Ag85A or Ag85B genes were synthesized with optimized codon usage for human cells. The genes were then independently flanked with the gene end (GE), intergenic region (I), and gene start (GS), which are important for viral mRNA synthesis [36]. The sequences were inserted between the HN and L genes of PIV5 creating plasmids pPIV5-85A or pPIV5-85B (Fig. 1A). Plasmids pPIV5-85A or pPIV5-85B (3 μg), along with plasmids pCAGGS-PIV5-L (1.5 μg), pCAGGS-PIV5-NP (1 μg), and pCAGGS-PIV5-P (200 ng), were transfected into

BSRT-7 cells. At 4 days post-transfection, supernatants containing PIV5-85A and PIV5-85B viruses were collected and plaques purified in BHK21 cells. Plaques (developing 4 to 7 days post-infection [dpi]) were selected and further amplified in MDBK cells. RNA was extracted from the supernatant using a QIAmp viral RNA minikit, and reverse transcription (RT) was performed with random primers. The reverse transcription product was further amplified by PCR using specific primers for viruses.

Growth curve and plaque assay

MDBK cells in 6-well plates were infected with viruses at a MOI of 0.1. The supernatants were collected everyday up to six days post-infection. Titers of viruses in the media were determined using palque assays in BHK21 as described before [27–31].

Western blotting

Virus-infected MDBK cells were lysed with whole-cell extraction buffer (WCEB) (50 mM Tris-HCl [pH 8], 280 mM NaCl, 0.5% NP-40, 0.2 mM EDTA, 2 mM EGTA, and 10% glycerol) [27]. The lysates were cleared by centrifugation at 4,000 rpm for 15 minutes using a tabletop centrifuge, and the supernatants were subjected to Western blotting as before [27–31].

Preparation of *M. bovis* BCG and *M. tuberculosis* cultures

M. bovis BCG Pasteur original culture was obtained from the laboratory of Dr. David Russell. Log-phase cultures of BCG in Middlebrook 7H9 medium, supplemented with 0.5% glycerol, 10% albumin-dextrose-catalase (ADC) and 0.05% Tween 80 (7H9gtADC), were frozen in aliquots at −70°C until needed. For immunizations, a vial of BCG was thawed and cultured in 7H9gtADC until the $OD_{600\ nm}$ reached 1.0. The cells were harvested by centrifugation, resuspended in phosphate-buffered -saline with 0.01% Tween 80 (PBS-T), passed through a tuberculin syringe to remove clumps, and counted on a Petroff Hauser Counter. Each mouse received 10 CFU subcutaneously (S.C.).

M. tuberculosis strain Erdman (Food and Drug Administration, USA) was grown from seed stocks in Proskauer-Beck liquid medium with 0.05% Tween 80 to log phase ($OD_{600\ nm}$ = 1.0) and frozen in aliquots at −70°C until needed. The titers were calculated as colony forming units (CFU)/mL by plating on Middlebrook 7H10 agar plates supplemented with 0.5% glycerol, 10% ADC, and 0.05% Tween 80 (7H10gtADC). Cultures were diluted in sterile PBS-T prior to use. The seed stocks were prepared by culturing *M. tuberculosis* Erdman as pellicles on Proskauer-Beck medium. To prepare the inoculum for aerosol infection, the frozen vials of *M. tuberculosis* Erdman were thawed, passed through a tuberculin syringe to remove clumps, and diluted in PBS-T. The titer was measured after plating on 7H10gtADC plates.

Animal vaccination and challenge studies

Six- to eight-week-old, female, BALB/c mice were used in the animal studies. All animal experiments were performed following protocols approved by the Institutional Animal Care and Use Committee, University of Georgia. For BCG prime immunization, BALB/c mice were first inoculated subcutaneously with 10 CFU of BCG. Seven weeks later, some of the

mice were boosted by intranasally dropping 100 μL PIV5-85A or PIV5-85B at dose of 10 PFU per mouse. The mouse groups were designated as prime-boost: PBS-PBS, PBS-PIV5-85A, PBS-PIV5-85B, BCG-PBS, BCG-PIV5-85A, and BCG-PIV5-85B.

The mice were challenged with 50–100 CFU of *M. tuberculosis* Erdman using a Madison aerosol chamber [37]. Unvaccinated mice were sacrificed 24 hour post-challenge to confirm actual exposure dose of *M. tuberculosis* to be 50 to 100 CFU in the lungs. All mice were monitored daily for malaise or other adverse reactions. Subcutaneous BCG-injected mice had their injection sites visually examined at first daily and then weekly.

After euthanasia, tissues including lungs, livers, and spleens were harvested and placed in PBS with 0.05% Tween 80 and homogenized. Serially-diluted homogenates was plated onto 7H10gtADC agar plates and incubated at 37°C for three weeks prior to colony count assessments.

ELISA

Immulon 2HB 96-well microtiter plates (Thermo Lab Systems) were coated with 0.1 μg of recombinant *M. tuberculosis* antigen 85A or 85B proteins (BEI Resources) and incubated at 4°C overnight. Assays were performed according to the manufacturer's instructions (KPL). Serial dilutions of serum samples from mice were added onto the antigen-coated plates. Goat anti-mouse IgG conjugated to HRP (KPL) was added, and the plates were developed. The optical density (OD) was measured at 450 nm with a Bio-Tek Powerwave XS plate reader. The IgG titer was determined to be the lowest serum dilution with an OD greater than that of the mean of naive serum plus two standard deviations.

IFN-γ ELISPOT assay

The spleens were collected and homogenized and washed with Hank's Balanced Salt Solution. Gey's solution was added to remove the red blood cells. Splenocytes in complete tumor medium were added to 96-well plates (BD Biosciences). The cells were mock stimulated or stimulated with *M. tuberculosis* antigen 85A or 85B proteins (BEI Resources), HIV gag peptide (as a control protein), or phorbol myristate acetate (PMA)-ionomycin. Cultures were incubated at 37°C and in 5% CO_2 for 48 hours. The plates were washed and incubated with antibody and substrate according to the manufacturer's instructions (BD Biosciences). The spots were counted using an AID ViruSpot Reader (Cell Technology). The results are presented as mean numbers of cytokine-secreting cells subtracted from the total number of mock-stimulated cells per 10 splenocytes.

Pathology

After euthanasia, lungs were collected, fixed in 10% neutral-buffered formalin, and processed for histopathology. The histologic slides were scored by a pathologist blinded to the identity of the individual experimental samples. Sections were assessed subjectively for percentage of the section affected by the presence of granulomas, then assessed quantitatively for the number of granulomas per section.

Data analysis

Comparisons were made using Student's *t* test and the Mann-Whitney test within the program GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Generation and analysis of PIV5 expressing *M. tuberculosis* antigen 85A or 85B

Infectious PIV5 expressing 85A (PIV5-85A) and 85B (PIV5-85) viruses were obtained from pPIV5-85A and pPIV5-85B (Fig. 1A) [31], plaque-purified and grown to large volumes. To confirm the insertion of 85A and 85B, the viruses were subjected to RT-PCR and PCR products corresponding to the expected sizes of insertions in PIV5-85A and PIV5-85B were detected (Fig. 1B, expected sizes of PIV5, PIV5-85A, PIV5-85B were 300 bp, 1,300 bp and 1,300 bp respectively), indicating that the 85A and 85B genes were inserted as planned Furthermore, the genomes of PIV5-85A and PIV5-85B were sequenced and matched the input cDNA sequences (data not shown).

To determine expression of *M. tuberculosis* antigen 85A or 85B in PIV5-85A and PIV5-85B-infected cells, lysates from infected cells were immunoblotted. *M. tuberculosis* Ag85A or Ag85B protein was detected in the PIV5-85A or PIV5-85B-infected cells, respectively, but not in PIV5-infected cells (Fig. 2).

Analysis of growth rates of PIV5-85A and PIV5-85B in tissue culture cells

Growth rates of viruses in MDBK cells were compared. Both PIV5-85A and PIV5-85B viruses displayed slightly delayed growth kinetics at 1–2 days post-infection compared to PIV5, but grew to similar titers by 4 days post-infection (Fig. 3).

Immune responses of mice vaccinated with PIV5-85A and PIV5-85B

To determine the immunogenicity of PIV5-85A and PIV5-85B, mice were prime-immunized with PBS or BCG (Fig. 4A). After 7 weeks, the PBS group was immunized with PBS, PIV5-85A, or PIV5-85B (PBS-PBS, PBS-PIV5-85A and PBS-PIV5-85B, respectively). Similarly, the BCG group was boosted with PBS, PIV5-85A, or PIV5-85B (BCG-PBS, BCG-PIV5-85A, and BCG-PIV5-85B, respectively). At 2 weeks post-boost vaccination, the mice were bled, and total serum IgG antibody titers to *M. tuberculosis* antigen 85A or 85B were measured (Fig. 4A). Mice immunized with BCG, PIV5-85A, or PIV5-85B developed *M. tuberculosis* Ag85A- or Ag85B-specific serum IgG antibodies (Fig. 4B and 4C). Mice vaccinated with BCG-PIV5-85A generated the highest titers of anti-*M. tuberculosis* Ag85A, and mice vaccinated BCG-PIV5-85B generated the highest anti-*M. tuberculosis* Ag85B antibody titers, indicating that BCG prime with PIV5- 85A or PIV5-85B synergized *M. tuberculosis* antigen-specific antibody production. To determine the effects of the immune sera on *M. tuberculosis*, we have performed Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) assay. We did not observe any killing of *M. tuberculosis* by these sera (data not shown), suggesting that the sera are not likely protective against *M. tuberculosis* infection.

Cellular immune responses were examined using an IFN-γ ELISPOT assay. Mice were sacrificed at 3 weeks after boost and splenocytes were analyzed. Compared to PBS-PBS-vaccinated mice, PBS-PIV5-85A, PBS-PIV5-85B, BCG-PBS, BCG-PIV5-85A, and BCG-PIV5-85B-vaccinated mice induced higher levels of *M. tuberculosis* Ag85A- or Ag85B-specific T cell responses, although the differences between some of the groups were not statistically significant. BCG-PIV5-85A and BCG-PIV5-85B groups generated higher levels of *M. tuberculosis* Ag85A- or Ag85B-specific cellular immune responses than prime-only groups, suggesting BCG prime and PIV5-Ag85A/85B boost enhance cell-mediated responses than BCG alone (Fig. 5).

Protective efficacy of PIV5-85A and PIV5-85B in mouse aerosol challenge model

At 10 weeks post-prime vaccination, mice were challenged with 50–100 CFU of *M. tuberculosis* strain Erdman using a Madison aerosol infection chamber. Four weeks and 9 weeks post-challenge, mice were sacrificed and organ bacterial loads assessed. Comparing to the PBS-PBS group, all of the vaccinations except PBS-PIV5-85B showed varied levels of protection against infection. Lungs from mice vaccinated with BCG-PIV5-85A or BCG-PIV5-85B contained the lowest CFU counts, indicating that either regimen conferred superior levels of protection against challenge (Fig. 6A and C). At 47 weeks after challenge, the BCG-PIV5-85A and BCG-PIV5-85B groups had the least numbers of CFUs in the lungs, while the PBS-PIV5-85A and PBS-PIV5-85B groups had comparable CFUs to the BCG group in the lungs, indicating that PIV5-85A or PIV5-85B alone were at least as effective as BCG in controlling *M. tuberculosis* replication in lungs, and PIV5-85A or PIV5-85B boost after BCG prime further reduced bacterial numbers in the lungs (Fig. 6E). At 4, 9, and 47 weeks post-*M. tuberculosis* infection, the BCG-PIV5-85A and BCG-PIV5-85B groups had consistently lower levels of bacterial load in the lungs than the PBS group whereas there were no differences between BCG and PBS group at 9 and 47 weeks post challenge. BCG-PIV5-85A and PIV5-85B group had lower number of bacteria in the lungs than that of BCG group, though the differences were not statistically significant. Furthermore, there were no significant differences in the bacterial load in the spleens of unimmunized versus immunized mice; however, there was at least a half-log reduction in bacterial counts in the spleens of the BCG-PIV5-85A and BCG- PIV5-85B groups at weeks 4, 9, and 47 post-*M. tuberculosis* infection (Fig. 6B, D, and F).

Pathology results in the lungs are consistent with bacterial load data. There was no difference in numbers of granulomas and areas affected by granulomas among groups at 9 weeks post-challenge (Fig. 7A and B). However, at 47 weeks after challenge, the PBS group had the most granulomas and the largest areas containing granulomatous masses, while PIV5-85A, PIV5-85B, and BCG-immunized groups had similar numbers of granulomas and areas containing granulomas (Fig. 7C). Groups primed with BCG and boosted withPIV5-85A or PIV5-85B had the least numbers of granulomas and the smallest areas containing granulomas (Fig. 7A, B and C).

DISCUSSION

Antigens from various viruses (Influenza, RSV, and rabies) have been previously expressed using PIV5, and these PIV5-vectored vaccines have been shown to be effective in protecting animals from the relevant infection [27–32, 38, 39]. In this study, for the first time, it has been demonstrated that PIV5, a vaccine vector based on a eukaryotic virus, expresses bacterial antigens that were protective.

The exact mechanism of the protection afforded by PIV5-based *M. tuberculosis* vaccine is not clear. While we have detected both humoral and cell-mediated immune responses, we did not observe killing of *M. tuberculosis* by the immune sera in an ADCC assay (data not shown), suggesting that antibody did not play a role in the protection. This is consistent with the fact that Ag85A and Ag85B are not surface proteins. Because of the detection of cell-mediated responses to Ag85A and Ag85B in PIV5-85A/85B immunized mice, we speculate that cell-mediated immune responses are responsible for the protection.

Recently, it was shown that PIV5-expressing influenza virus HA protein with insertion of the H5N1 HA coding region between the PIV5 SH and HN genes generates superior immune responses and protection compared to inserting the HA coding region between HN and L genes [28]. In this study, the *M. tuberculosis* Ag85A or Ag85B coding regions were inserted between the PIV5 HN and L genes, and although expression of the antigens and protective levels of anti-*M. tuberculosis* immune responses were achieved, further exploration of insertion site preferences may improve antigen expression, immunogenicity, and protective efficacy of the PIV5-85A and PIV5-85B candidates. Furthermore, it has been found that modification of the PIV5 genome can enhance immune responses and protection afforded by PIV5-based vaccines [30]. The SH protein of PIV5 is known to play a role in blocking TNF-α signaling, and PIV5 lacking SH induces expression of TNF-α [40–42]. Thus, PIV5 not expressing SH is a better vector than wild-type PIV5 for expressing HA of the H5N1 influenza virus [30]. The same approach can be explored to improve the efficacy of PIV5-based *M. tuberculosis* vaccines.

The murine aerosol challenge model has been extensively utilized for preliminary *M. tuberculosis* vaccine efficacy testing. It is relatively inexpensive and the genetic background of the test animals is homogeneous. However, the mouse is not a good model to study *M. tuberculosis* infection and pathogenesis, and thus, if a vaccine candidate is shown to be as protective as BCG in the mouse model, more complex models, including the guinea pig, bovine, and most importantly, non-human primate are subsequently examined [43]. One interesting aspect of PIV5 is that it replicates more efficiently in non-human primates than in mice (data not shown). This is likely due to the fact that PIV5 encodes a viral protein, V, that can block interferon signaling in non-human primates but not in mouse cells, because V can cause degradation of STAT1 in NHP cells but not in mouse cells [44]. In our experience, for a live vaccine based on a live viral vector, the better the replication, the more efficacious the vaccine [31, 39]. Thus, it is likely that PIV5-based vaccines will generate more robust immune responses in non-human primates (and humans) than in mice.

Acknowledgments

The authors thank all the members of B.H. and F.Q. laboratories for their helpful discussion and technical assistance. In particular, the authors thank Monica LaGatta, Kim Goldbach, Simon Owino and Shelly Helms for their assistance with the aerosol infections and animal care. This work was supported by a grant from the National Institute of Allergy and Infectious Disease (R21AI103575) to B.H.

References

1. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA. 1994; 271:698–702. [PubMed: 8309034]
2. Fine PE. Variation in protection by BCG: implications of and for heterologous immunity. Lancet. 1995; 346:1339–45. [PubMed: 7475776]
3. Mitchison DA. The diagnosis and therapy of tuberculosis during the past 100 years. Am J Respir Crit Care Med. 2005; 171:699–706. [PubMed: 15709048]
4. Dye C, Watt CJ, Bleed DM, Hosseini SM, Raviglione MC. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. JAMA: the journal of the American Medical Association. 2005; 293:2767–75. [PubMed: 15941807]
5. Castanon-Arreola M, Lopez-Vidal Y. A second-generation anti TB vaccine is long overdue. Ann Clin Microbiol Antimicrob. 2004; 3:10. [PubMed: 15176980]
6. McMurray DN. Recent progress in the development and testing of vaccines against human tuberculosis. Int J Parasitol. 2003; 33:547–54. [PubMed: 12782054]
7. Brennan MJ, Fruth U. Global Forum on TB Vaccine Research and Development. World Health Organization, June 7–8 2001, Geneva. Tuberculosis (Edinb). 2001; 81:365–8. [PubMed: 11800588]
8. Flynn JL. Immunology of tuberculosis and implications in vaccine development. Tuberculosis (Edinb). 2004; 84:93–101. [PubMed: 14670350]
9. Besnard M, Sauvion S, Offredo C, Gaudelus J, Gaillard JL, Veber F, et al. Bacillus Calmette-Guerin infection after vaccination of human immunodeficiency virus-infected children. The Pediatric infectious disease journal. 1993; 12:993–7. [PubMed: 8108227]
10. Talbot EA, Perkins MD, Silva SF, Frothingham R. Disseminated bacille Calmette-Guerin disease after vaccination: case report and review. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 1997; 24:1139–46. [PubMed: 9195072]
11. Sieling PA, Torrelles JB, Stenger S, Chung W, Burdick AE, Rea TH, et al. The human CD1-restricted T cell repertoire is limited to cross-reactive antigens: implications for host responses against immunologically related pathogens. Journal of immunology. 2005; 174:2637–44.
12. Ulrichs T, Moody DB, Grant E, Kaufmann SH, Porcelli SA. T-cell responses to CD1-presented lipid antigens in humans with Mycobacterium tuberculosis infection. Infect Immun. 2003; 71:3076–87. [PubMed: 12761085]
13. Gupta UD, Katoch VM. Animal models of tuberculosis for vaccine development. Indian J Med Res. 2009; 129:11–8. [PubMed: 19287053]
14. Kita Y, Okada M, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, et al. Development of therapeutic and prophylactic vaccine against Tuberculosis using monkey and transgenic mice models. Hum Vaccin. 2011; 7(Suppl):108–14. [PubMed: 21263229]
15. Verreck FA, Vervenne RA, Kondova I, van Kralingen KW, Remarque EJ, Braskamp G, et al. MVA. 85A boosting of BCG and an attenuated, phoP deficient M. tuberculosis vaccine both show protective efficacy against tuberculosis in rhesus macaques. PLoS One. 2009; 4:e5264. [PubMed: 19367339]
16. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet. 2013; 381:1021–8. [PubMed: 23391465]
17. Lamb, RA., Kolakofsky, D. *Paramyxoviridae*: The viruses and their replication. In: Knipe, DM., Howley, PM., editors. Fields Virology. 4. Philadelphia: Lippincott, Williams and Wilkins; 2001.

18. Binn LN, Eddy GA, Lazar EC, Helms J, Murnane T. Viruses recovered from laboratory dogs with respiratory disease. Proc Soc Exp Biol Med. 1967; 126:140–5. [PubMed: 4294430]
19. Rosenberg FJ, Lief FS, Todd JD, Reif JS. Studies of canine respiratory viruses. I. Experimental infection of dogs with an SV5-like canine parainfluenza agent. Am J Epidemiol. 1971; 94:147–65. [PubMed: 4327998]
20. Cornwell HJ, McCandlish IA, Thompson H, Laird HM, Wright NG. Isolation of parainfluenza virus SV5 from dogs with respiratory disease. The Veterinary record. 1976; 98:301–2. [PubMed: 179186]
21. McCandlish IA, Thompson H, Cornwell HJ, Wright NG. A study of dogs with kennel cough. Vet Rec. 1978; 102:293–301. [PubMed: 207006]
22. Azetaka M, Konishi S. Kennel cough complex: confirmation and analysis of the outbreak in Japan. Nippon Juigaku Zasshi. 1988; 50:851–8. [PubMed: 2845177]
23. Kontor EJ, Wegrzyn RJ, Goodnow RA. Canine infectious tracheobronchitis: effects of an intranasal live canine parainfluenza-Bordetella bronchiseptica vaccine on viral shedding and clinical tracheobronchitis (kennel cough). Am J Vet Res. 1981; 42:1694–8. [PubMed: 6275747]
24. He B, Paterson RG, Ward CD, Lamb RA. Recovery of infectious SV5 from cloned DNA and expression of a foreign gene. Virology. 1997; 237:249–60. [PubMed: 9356337]
25. Zhang L, Collins PL, Lamb RA, Pickles RJ. Comparison of differing cytopathic effects in human airway epithelium of parainfluenza virus 5 (W3A), parainfluenza virus type 3, and respiratory syncytial virus. Virology. 2011; 421:67–77. [PubMed: 21986028]
26. Arimilli S, Alexander-Miller MA, Parks GD. A simian virus 5 (SV5) P/V mutant is less cytopathic than wild-type SV5 in human dendritic cells and is a more effective activator of dendritic cell maturation and function. J Virol. 2006; 80:3416–27. [PubMed: 16537609]
27. Phan SI, Chen Z, Xu P, Li Z, Gao X, Foster SL, et al. A respiratory syncytial virus (RSV) vaccine based on parainfluenza virus 5 (PIV5). Vaccine. 2014; 32:3050–7. [PubMed: 24717150]
28. Li Z, Mooney AJ, Gabbard JD, Gao X, Xu P, Place RJ, et al. Recombinant Parainfluenza Virus 5 Expressing Hemagglutinin of Influenza A Virus H5N1 Protected Mice against Lethal Highly Pathogenic Avian Influenza Virus H5N1 Challenge. Journal of virology. 2013; 87:354–62. [PubMed: 23077314]
29. Li Z, Gabbard JD, Mooney A, Gao X, Chen Z, Place RJ, et al. SIngle Dose Vaccination of A Recombinant Parainfluenza Virus 5 Expressing NP from H5N1 Provides Broad Immunity Against Influenza A VIruses. Journal of Virology. 2013; 87
30. Li Z, Gabbard JD, Mooney A, Chen Z, Tompkins SM, He B. Efficacy of Parainfluenza virus 5 mutants expressing HA from H5N1 influenza A virus in mice. Journal of Virology. 2013
31. Chen Z, Zhou M, Gao X, Zhang G, Ren G, Gnanadurai CW, et al. A novel rabies vaccine based on a recombinant parainfluenza virus 5 expressing rabies virus glycoprotein. J Virol. 2013; 87:2986–93. [PubMed: 23269806]
32. Chen Z, Xu P, Salyards GW, Harvey SB, Rada B, Fu ZF, et al. Evaluating a Parainfluenza Virus 5-Based Vaccine in a Host with Pre-Existing Immunity against Parainfluenza Virus 5. PLoS One. 2012; 7:e50144. [PubMed: 23185558]
33. Price GE, Soboleski MR, Lo CY, Misplon JA, Quirion MR, Houser KV, et al. Single-dose mucosal immunization with a candidate universal influenza vaccine provides rapid protection from virulent H5N1, H3N2 and H1N1 viruses. PLoS One. 2010; 5:e13162. [PubMed: 20976273]
34. Sekaly RP. The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? The Journal of experimental medicine. 2008; 205:7–12. [PubMed: 18195078]
35. Lawson CM, Bennink JR, Restifo NP, Yewdell JW, Murphy BR. Primary pulmonary cytotoxic T lymphocytes induced by immunization with a vaccinia virus recombinant expressing influenza A virus nucleoprotein peptide do not protect mice against challenge. Journal of virology. 1994; 68:3505–11. [PubMed: 7514677]
36. He B, Lamb RA. Effect of inserting paramyxovirus simian virus 5 gene junctions at the HN/L gene junction: analysis of accumulation of mRNAs transcribed from rescued viable viruses. J Virol. 1999; 73:6228–34. [PubMed: 10400712]

37. Bertholet S, Ireton GC, Kahn M, Guderian J, Mohamath R, Stride N, et al. Identification of human T cell antigens for the development of vaccines against Mycobacterium tuberculosis. J Immunol. 2008; 181:7948–57. [PubMed: 19017986]

38. Tompkins SM, Lin Y, Leser GP, Kramer KA, Haas DL, Howerth EW, et al. Recombinant parainfluenza virus 5 (PIV5) expressing the influenza A virus hemagglutinin provides immunity in mice to influenza A virus challenge. Virology. 2007; 362:139–50. [PubMed: 17254623]

39. Mooney AJ, Li Z, Gabbard JD, He B, Tompkins SM. Recombinant Parainfluenza Virus 5 Vaccine Encoding the Influenza Virus Hemagglutinin Protects against H5N1 Highly Pathogenic Avian Influenza Virus Infection following Intranasal or Intramuscular Vaccination of BALB/c Mice. Journal of virology. 2013; 87:363–71. [PubMed: 23077318]

40. Wilson RL, Fuentes SM, Wang P, Taddeo EC, Klatt A, Henderson AJ, et al. Function of Small Hydrophobic Proteins of Paramyxovirus. J Virol. 2006; 80

41. Lin Y, Bright AC, Rothermel TA, He B. Induction of apoptosis by paramyxovirus simian virus 5 lacking a small hydrophobic gene. J Virol. 2003; 77:3371–83. [PubMed: 12610112]

42. He B, Leser GP, Paterson RG, Lamb RA. The paramyxovirus SV5 small hydrophobic (SH) protein is not essential for virus growth in tissue culture cells. Virology. 1998; 250:30–40. [PubMed: 9770417]

43. Rowland R, McShane H. Tuberculosis vaccines in clinical trials. Expert Rev Vaccines. 2011; 10:645–58. [PubMed: 21604985]

44. Young DF, Chatziandreou N, He B, Goodbourn S, Lamb RA, Randall RE. Single amino acid substitution in the V protein of simian virus 5 differentiates its ability to block interferon signaling in human and murine cells. J Virol. 2001; 75:3363–70. [PubMed: 11238862]

HIGHLIGHT

We developed a novel TB vaccine that is as effective as BCG in mice.

Using BCG-prime and novel-vaccine boost, we further enhanced efficacy of the vaccine candidates.

We have for the first time shown that PIV5 is effective as a vaccine vector for bacteria pathogen.

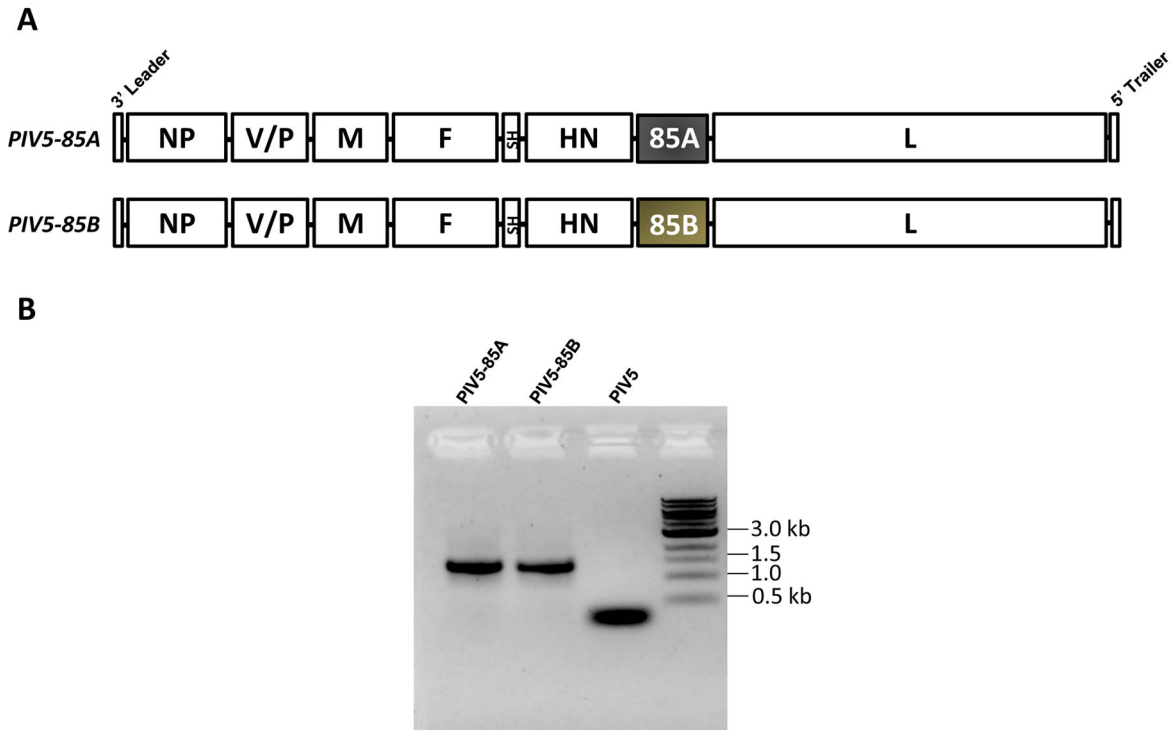


FIG. 1. Generation of PIV5-85A or PIV5-85B viruses

(A) Schematics of recombinant PIV5 viruses expressing *M. tuberculosis* antigen 85A and 85B proteins. The PIV5 genome contains seven genes in the order of 3′-NP-V/P-M-F-SH-HN-L-5′ with leader and trailer regions located at the ends of the genome. The *M. tuberculosis* antigen 85A or 85B gene was inserted into PIV5 between the HN and L genes. (B) After virus plaque purification, viral RNA was extracted and subjected to RT-PCR to confirm correct insertion and orientation. PIV5 alone served as the negative control.

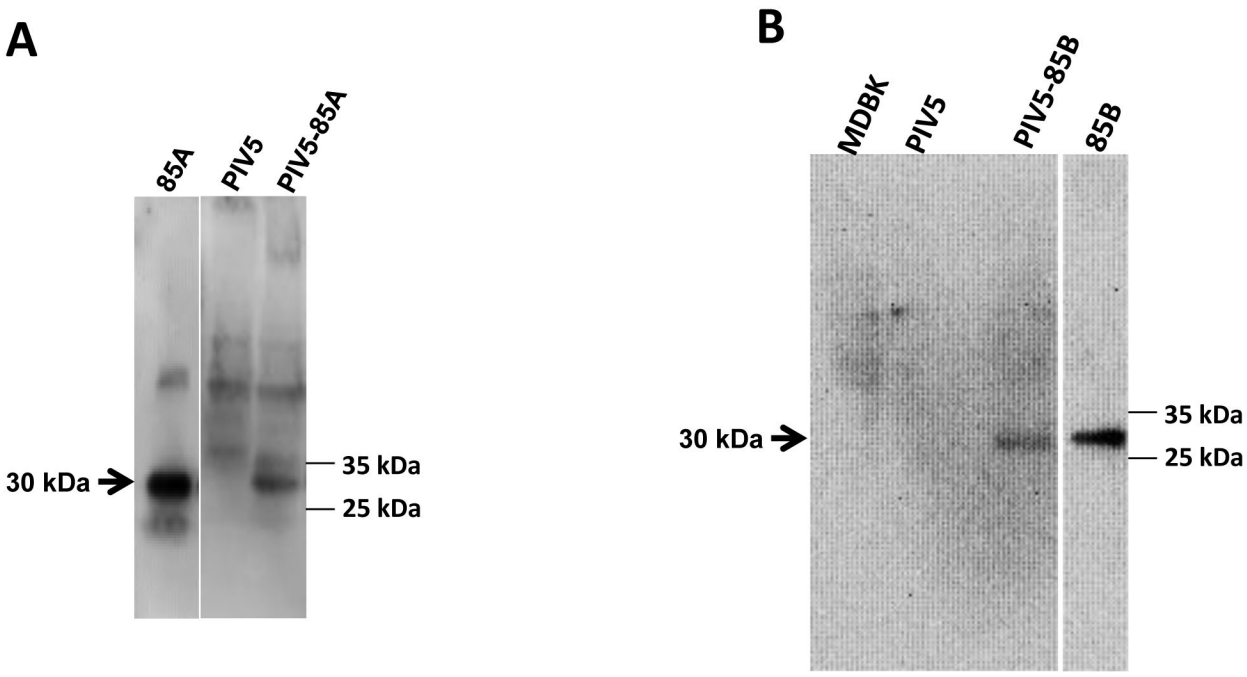


FIG. 2. Detection of *M. tuberculosis* Ag85A or Ag85B expression by western blot

Lysates prepared from MDBK cells infected with PIV5, PIV5-85A, PIV5-85B, or mock-infected were immunoblotted with anti-*M. tuberculosis* Ag85A or Ag85B antibodies. *M. tuberculosis* Ag85A and Ag85B proteins served as positive controls. Ag85A or Ag85B protein is only detected in lysates from cells infected with the antigen-encoding PIV5 derivatives.

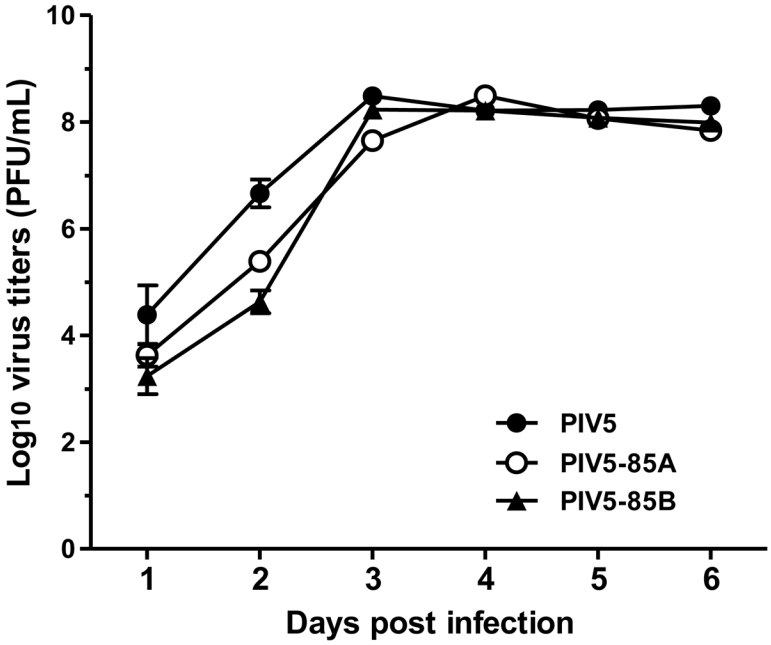


FIG. 3. Comparison of growth kinetics of PIV5-85A, PIV5-85B, and PIV5

Multi-cycle growth curves of PIV5, PIV5-85A, and PIV5-85B were performed in MDBK cells at MOI = 0.1. Aliquots of supernatant from the cell culture infections were harvested at 24-hour intervals. The virus titers in the aliquots were determined by plaque assay in BHK21 cells. Values represent averages from two independent experiments. Error bars indicate standard deviation.

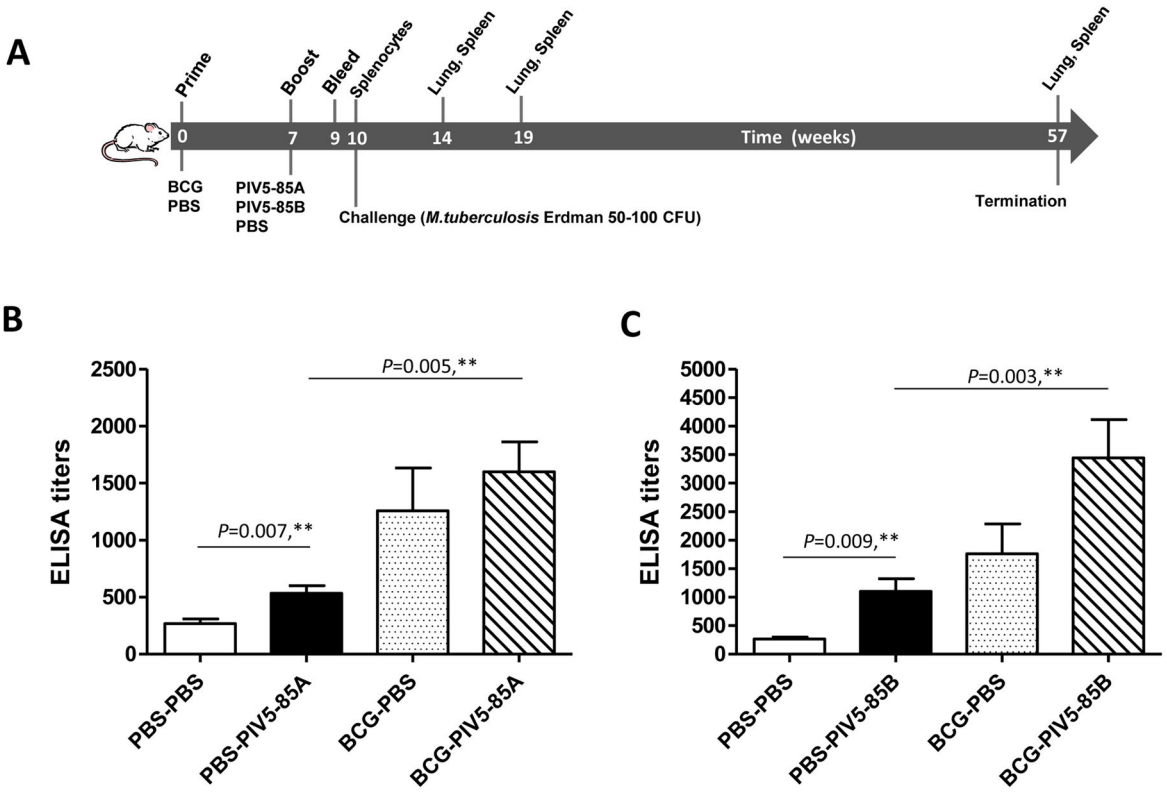


FIG. 4. Determination of antibody responses in vaccinated animals

(A) Schematic diagram of animal experimental design. Mice were vaccinated (s.c.) with BCG or phosphate-buffered saline (PBS) at week 0, and boosted (intranasally) with PIV5-85A, PIV5-85B, or PBS at week 7. (B) ELISA antibody titer against M. tuberculosis Ag85A. BALB/c mice were bled at week 9 post-prime immunization. *M. tuberculosis* Ag85A -specific IgG antibody titer in serum samples was measured in 85A protein-coated plates by ELISA. (C) ELISA antibody titer against *M. tuberculosis* Ag85B. BALB/c mice were bled at week 9 post-prime immunization. *M. tuberculosis* Ag85B -specific IgG antibody titer in serum samples was measured in 85B protein-coated plates by ELISA. The IgG titer was determined to be the lowest serum dilution with an OD greater than that of the mean of naive serum plus two standard deviations. Differences were evaluated by Student's t test. (*, $P < 0.05$; **, $P < 0.01$).

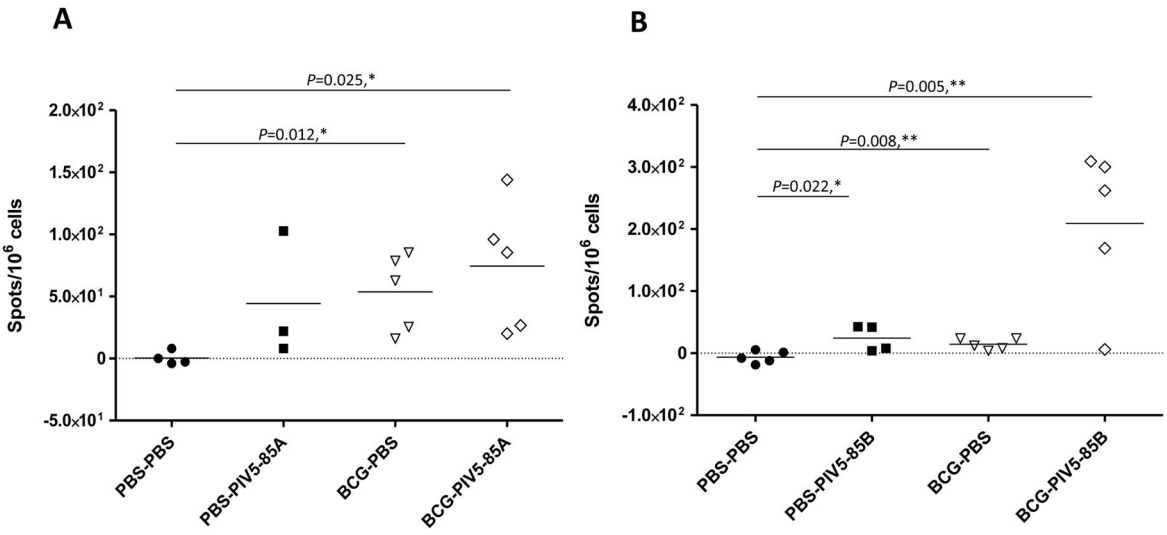
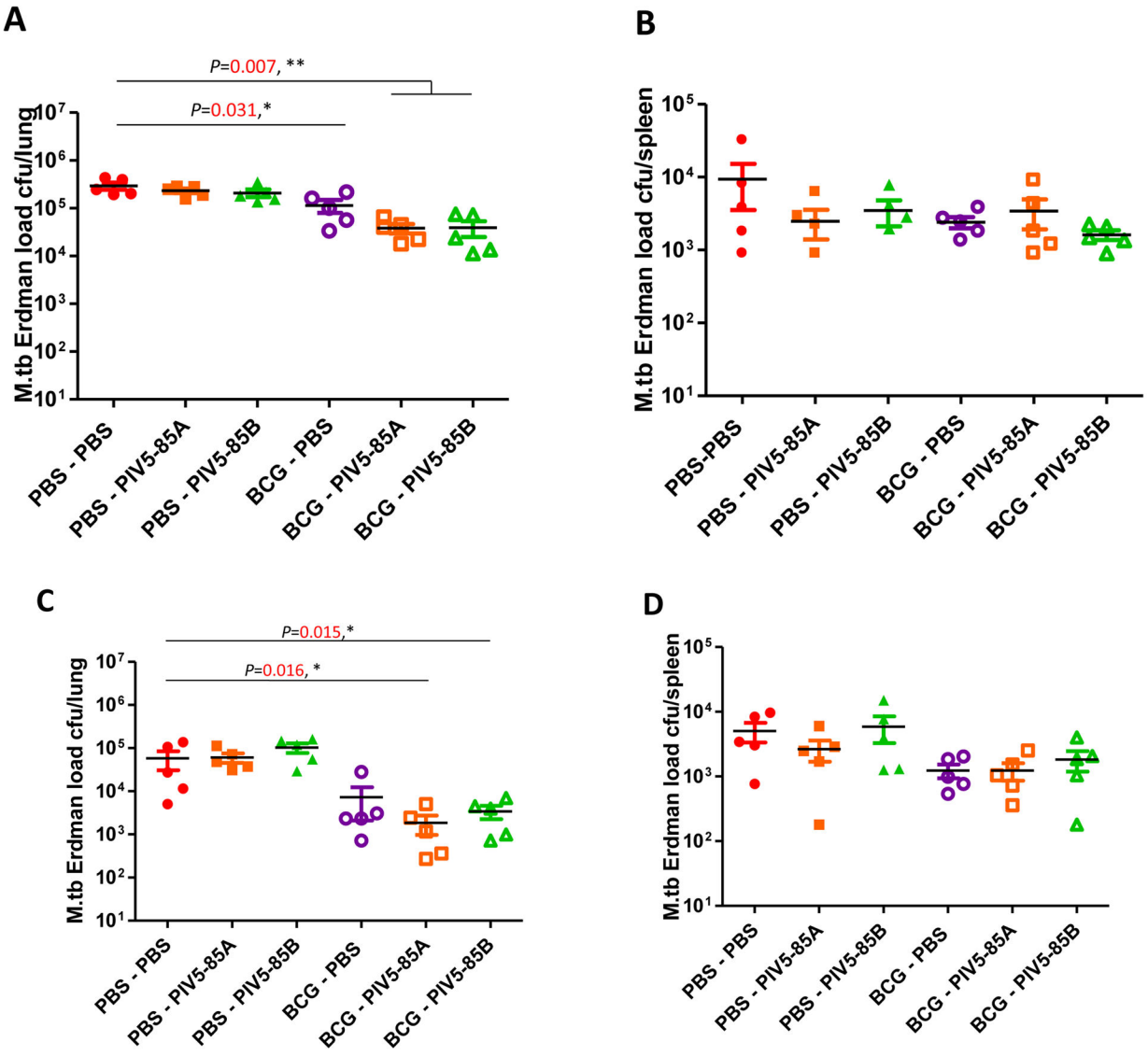


FIG. 5. Determination of cellular immune responses using ELISPOT

Mice were vaccinated with BCG and PIV5-85A or PIV5-85B in a prime-boost immunization regimen. (A) ELISPOT assay using Ag85A. At week 10 post-prime immunization, mice were sacrificed, and spleens were collected. Splenocytes were stimulated with M. tuberculosis Ag85A. (B) ELISPOT assay using Ag85B. At week 10 post-prime immunization, mice were sacrificed, and spleens were collected. Splenocytes were stimulated with M. tuberculosis Ag85B. Results are presented as the mean number of IFN-γ-producing cells per 10 splenocytes. Differences were evaluated by Student's *t* test. (*, $P < 0.05$; **, $P < 0.01$).



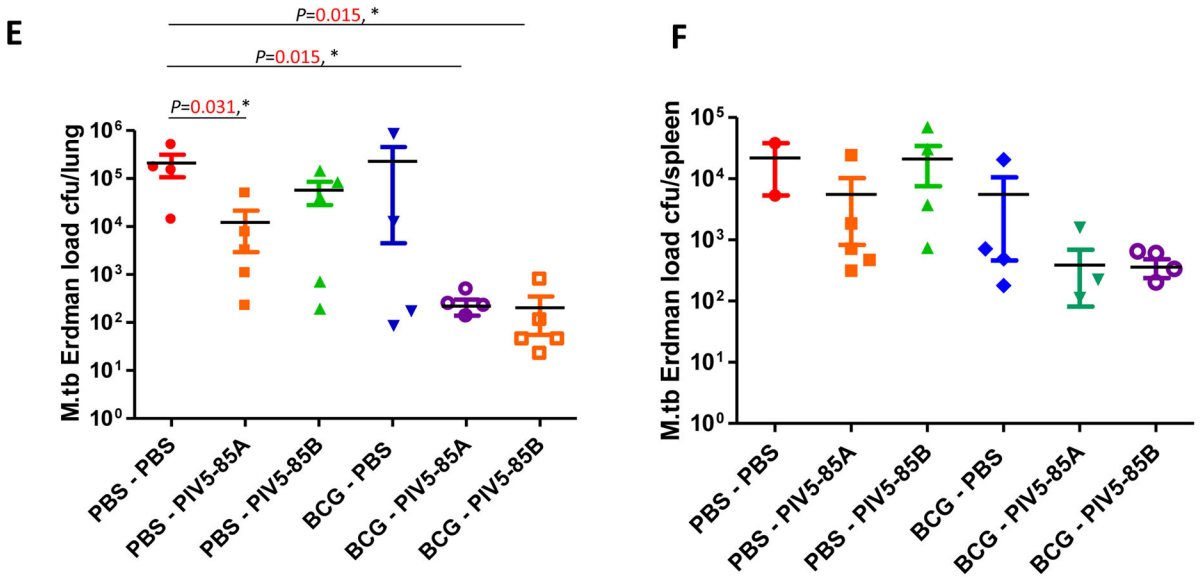


FIG. 6. Protective efficacy of PIV5-85A and PIV5-85B in mouse aerosol challenge model

The number of *M. tuberculosis* CFU in infected lungs (A,C,E) and spleens (B,D,F) was determined by colony enumeration assays. (A, B) 4 weeks post-challenge; (C,D) 9 weeks post-challenge; and (E,F) 47 weeks post-challenge. Data are presented as the means ± SEM of the results. Differences were evaluated by Mann-Whitney test. (*, $P < 0.05$; **, $P < 0.01$).

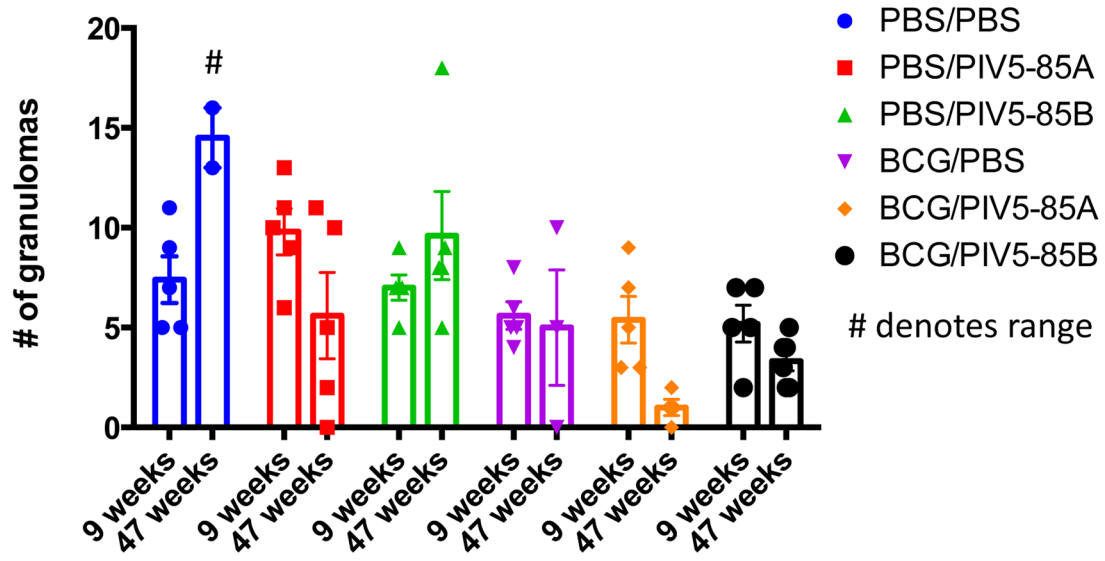


Fig. 7A

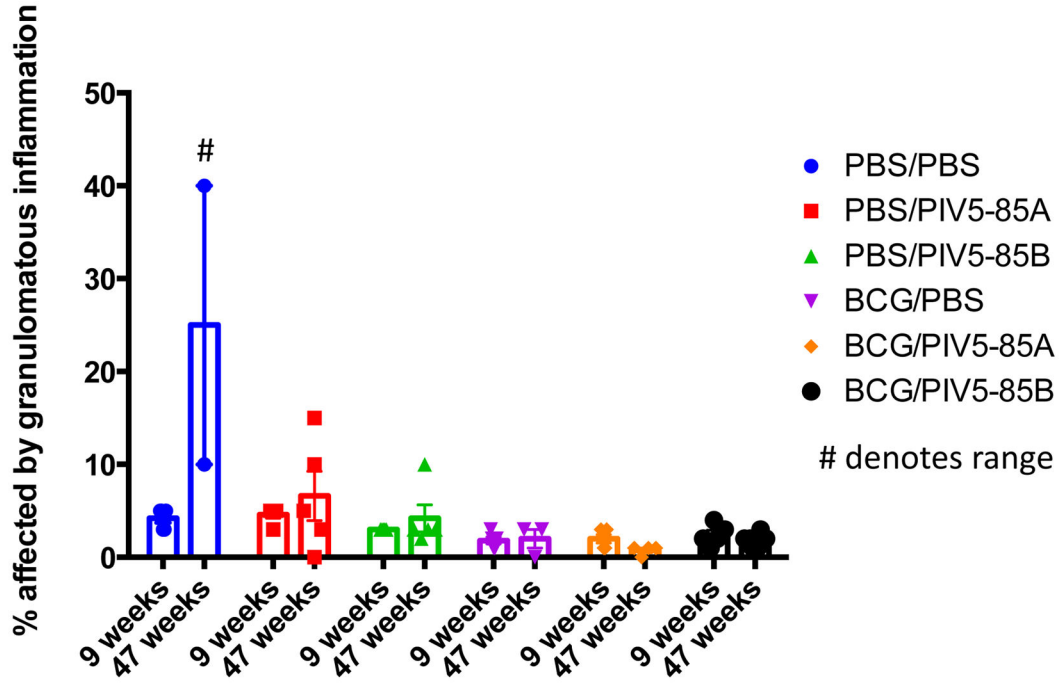


Fig. 7B

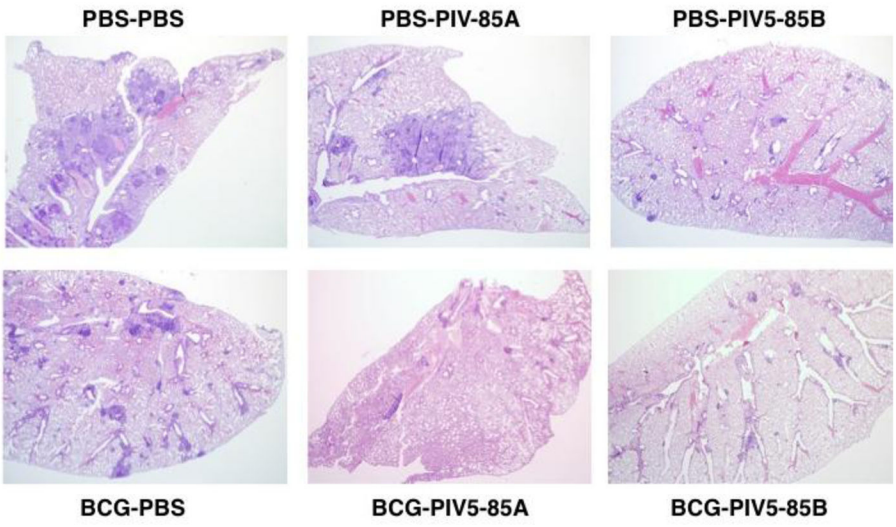


Fig. 7C

FIG. 7. Pathology of the lungs after challenge

(A) Number of granulomas in the lung sections. Due to attrition of animals, the PBS group and BCG group had only 2 and 3 mice, respectively, at the time of termination of the experiment (47 weeks after challenge). Thus, the formulation of statistically significant data was not possible. (B) Areas affected by granulomas. (C) Photomicrographs of lung sections. Pictures of representative lung sections from mice immunized with PBS, PIV5-85A, PIV5-85B, BCG, or primed with BCG and boosted with PIV5-85A or PIV5-85B at 2X magnification are shown.