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BCG vaccination in the cotton rat (*Sigmodon hispidus*) infected by the pulmonary route with virulent *Mycobacterium tuberculosis*

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Abstract

To evaluate the usefulness of the American cotton rat (*Sigmodon hispidus*) in the evaluation of vaccine-induced resistance, we infected BCG-vaccinated and non-vaccinated cotton rats with *Mycobacterium tuberculosis* (H37Rv) via the respiratory route. Lung histopathology of these animals showed loose, disorganized granulomas which were non-necrotic up to 8 weeks post-infection. Moreover, we were not able to detect a DTH response after intradermal injection with PPD antigen. Prior BCG vaccination significantly reduced lung and spleen bacterial loads by 1-1.5 log CFU and upregulated PPD-induced proliferation and production of IFN γ in lymphocyte cultures. We conclude that pulmonary infection of the cotton rat with *Mtb* more closely resembles the phenotype seen in mice rather than guinea pigs.

Keywords

Vaccine; cotton rat; tuberculosis

INTRODUCTION

Despite the fact that no single animal species used to study tuberculosis (TB) represents a fully ideal disease model, important experimental questions related to pathogenesis, virulence factors, and the host immune response will be addressed effectively only by using such models [1]. Furthermore, novel vaccine candidates and new anti-tuberculosis drugs can be screened effectively only using whole animals [1-3]. Each of the commonly used animal models of TB contribute important unique insights into the host-pathogen interaction. For example, a recent study demonstrated that both overlapping and unique sets of

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mycobacterial genes were responsible for mycobacterial survival and persistence in the tissues of mice and guinea pigs following pulmonary exposure to identical pools of mutants [4]. This finding demonstrates that a complete understanding of TB pathogenesis may come only from a comparative approach in which both current and new animal models are involved. Thus, the development and characterization of new animal models of TB which may provide a novel perspective on pathogenesis are important research priorities.

The American Cotton Rat (*Sigmodon hispidus* and *Sigmodon fulviventer*) has been used extensively to study a wide array of infectious diseases, including those caused by viruses (e.g. adenovirus, poliovirus, respiratory syncytial virus, influenza virus, herpes simplex virus, etc.), bacteria (e.g. *Borrelia*, *Francisella*, *Haemophilus*), fungi (e.g. *Microsporium*, *Trichophyton*), and parasites (e.g. *Coccidia*, *Fasciola*, *Leishmania*, and *Toxoplasma*) [5-8]. The availability of an impressive array of commercial reagents, including cytokines, chemokines, cell surface markers and monoclonal antibodies directed at these proteins, further contributes to the potential usefulness of the cotton rat as a novel model for the study of TB [5,9].

Recently, Elwood et al used both *S. hispidus* and *S. fulviventer* to study primary and latent infection with *Mycobacterium tuberculosis* (*Mtb*) [10,11]. These investigators described granulomatous lesions with central necrosis in the lungs, spleens and lymph nodes of cotton rats infected either by the intranasal or aerosol routes with *Mtb* H37Rv, and positive skin test responses to purified protein derivative (PPD) injected intradermally. [10]. Moreover, latent tuberculosis infection was reportedly developed in these animals at 10 months post intranasal infection[11]. The authors suggested that the cotton rat might prove useful as a new animal model for the study of pulmonary TB and, in particular, might fill a niche between the mouse and the guinea pig as a unique species in which to study novel TB vaccines as the cotton rat appeared to combine the relevant pathological features of TB in the guinea pig with many of the practical advantages of the mouse.

Therefore, we conducted a series of experiments designed to characterize the cotton rat as an animal model for the study of vaccine-induced protection against pulmonary TB. The primary objectives of this study were: 1) to establish the infectious dose necessary to produce uniform pulmonary and extrapulmonary mycobacterial disease in the cotton rat; 2) to evaluate the ability of BCG vaccination to elicit a protective response following aerosol challenge and; 3) to describe the histological appearance of the granulomatous response in vaccinated and non-vaccinated cotton rats. *In vitro* and *in vivo* measures of vaccine-induced cell mediated immunity including dermal tuberculin reactivity, and PPD-induced lymphoproliferation and IFN γ levels also were examined.

METHODS

Experimental animals

Inbred, male and female cotton rats (*Sigmodon hispidus*, Virion Systems, Inc., Rockville, MD) were used in all experiments. Animals were housed individually in polycarbonate caging with stainless steel grid floors and feeders, provided commercial rat chow and fresh water *ad libitum*, and maintained at 25°C on a 12 hour light:dark cycle. The animals were rested and allowed to acclimate for a period of 7-10 days before beginning any experiment. All animal procedures were reviewed and approved by the Texas A&M Institutional Animal Care and Use Committee (IACUC).

BCG vaccination and PPD skin test

Eight weeks prior to aerosol challenge, cotton rats were vaccinated subcutaneously with 10⁴ colony forming units (CFU) of *M. bovis* BCG 1331 (Statens Serum Institute, Copenhagen,

Denmark) in the left inguinal region. The freeze-dried vaccine was reconstituted in Sauton SSI diluent immediately prior to vaccination.

To measure delayed type hypersensitivity (DTH), cotton rats were injected intradermally with 5 µg/0.1 ml (250 TU) of PPD (Mycos Research, LLC, Loveland, CO). A shaved site on the animal's back was chosen for the injection of PPD. Skin test reactions were evaluated at 24, 48 and 72 hours following PPD injection and recorded as the mean of two perpendicular measurements of induration (mm).

Aerosol challenge and necropsy

Cultures of *Mycobacterium tuberculosis* H37Rv (ATCC 27294) were prepared and stored as single cell suspensions at -80°C according to a modification of Grover et al [12]. Prior to aerosol challenge, a vial was thawed rapidly, vortexed vigorously and sonicated with an Ultrasonics sonicator (Heat Systems-Ultrasonics, Inc., Plainview, NY) for 45-60 sec at an output setting of 8.0 to disrupt bacterial clumps. Following dilution in sterile saline, the mycobacterial suspension was introduced into the nebulizer of a Madison Aerosol Exposure Chamber (University of Wisconsin Engineering Shops, Madison, WI), according to published protocols [13,14]. In some experiments, the total number of mycobacteria implanted in the lungs was determined by homogenizing the entire lung one day post-infection and inoculating the entire homogenate onto plates of 7H11 Selective Media (BVA Scientific, San Antonio, TX).

At several intervals post-infection, cotton rats were euthanized with an overdose of sodium pentobarbital (Sleepaway™, Fort Dodge Animal Health, IA). To determine bacillary loads in the tissues, the right lower lobe of the lung and 2/3rds of the spleen were removed aseptically, homogenized in sterile saline, diluted appropriately, and inoculated onto Middlebrook 7H10 agar plates (BVA Scientific, San Antonio, TX). Colony-forming units (CFU) were enumerated following incubation at 37°C for 21 days and expressed as a mean $\log_{10} \pm \text{SEM}$ /tissue sample [14]. The minimal detectable limits were $\log_{10} 1.35$ and 1.0 in the lung and spleen, respectively.

For histopathological analysis, the left lower lung lobe was preserved in 10% neutral buffered formalin, processed and stained with hematoxylin and eosin, and examined by a trained pathologist (BRW) in a blinded fashion.

Lymphoproliferation

Cotton rat spleens were removed aseptically and gently homogenized in RPMI 1640 medium, pH 7.2, containing L-glutamine, HEPES (Gibco Invitrogen), penicillin (100 units/ml), streptomycin (100 µg/ml), 2-mercaptoethanol (0.01 mM), and 10% fetal bovine serum. Single cell suspensions were prepared according to our previously published protocol, with modifications as described below [13]. Viable splenocytes were enumerated by trypan blue exclusion and adjusted to a final concentration of 5×10^6 cells/ml. Cell viability was routinely 90-95%.

Cotton rat splenocytes (5×10^5 cells/0.1 ml) were cultured in 96-well plates (BD Falcon, Franklin Lakes, NJ) in the presence of a non-specific T cell mitogen (ConA; 5 µg/ml); a mycobacterial antigen (PPD; 12.5 µg/ml), or medium alone as a negative control for 72 hours at 37°C in a 5% CO₂ in air atmosphere [8]. [³H]-thymidine (MP Biomedicals, Santa Ana, CA) was added at a concentration of 1 µCi/well for the final 18 hours of culture. Cells were harvested onto glass fiber filters and proliferation, as measured by counts per minute (cpm) of incorporated radionucleotide, was quantified in a liquid scintillation counter (LS8000; Beckman Instruments, Inc., Fullerton, CA). The results were expressed as a

stimulation index (SI), which was calculated by dividing the average cpm of cells stimulated with mitogen or antigen by the average cpm of unstimulated cells.

Detection of IFN γ

Spleen cells were cultured as described above in the presence of mitogen (ConA, 5 γ g/ml), antigen (PPD, 12.5 γ g/ml) or medium alone (negative control) for 24 and 48 hours at 37°C in a 5% CO₂ in air atmosphere. Following *in vitro* stimulation, cell supernatants were collected and assayed for IFN γ protein using a commercially available cotton rat IFN γ sandwich ELISA kit according to the manufacturer's protocol (R&D Systems, Minneapolis, MN).

Statistical analysis

Data were expressed as means $\pm$ standard error of the mean (SEM). Where appropriate, the data were analyzed using either a Student's t-test or one-way ANOVA followed by Tukey's post-test for between-mean comparisons. A probability of $p < 0.05$ was considered statistically significant.

RESULTS

Mycobacterial infection

Initially, we evaluated the ability of two concentrations of *Mtb* H37Rv (10^6 and 10^7 CFU/ml introduced into the nebulizer) to produce uniform pulmonary infection in the cotton rat. These concentrations are 10-fold and 100-fold, respectively, higher than that necessary to establish progressive pulmonary and disseminated infection in the guinea pig. At intervals of 2, 4, 6, and 8 weeks post-infection, we quantified the bacillary loads in both lungs and spleens. The results demonstrated that neither nebulizer concentration (Fig. 1A: 10^6 CFU/ml; Fig. 1B: 10^7 CFU/ml) was sufficient to cause uniform pulmonary and extrapulmonary infection in cotton rats as there were a number of animals with no evidence of infection in the lung or spleen (Fig. 1). The animals from which viable bacilli were recovered from the lungs had low bacillary loads ranging from 2-3 log CFU at all time intervals tested (Fig 1A). Moreover, bacilli were detected in the spleens of only 2 of 4 cotton rats at 8 weeks post-infection with the low dose, suggesting that dissemination may not have occurred. Spleens from the cotton rats at 2, 4, and 6 weeks with post-infection with the low dose had no recoverable bacilli (Fig. 1A). At 8 weeks post-infection, 2 out of 4 of the spleens had detectable bacilli growth. When the concentration in the nebulizer suspension was increased to 10^7 CFU/ml, bacillary loads from the lungs of cotton rats were 10-fold higher (3-4.5 log CFU) reflecting the higher aerosol infection, and slightly more uniform as only one animal had undetectable bacillary growth (Fig. 1B). However, there was no change in lung bacillary loads during the 8 week follow-up study. At the 10^7 CFU/ml infection dose, bacillary loads were detectable in the spleens as early as 4 weeks post-infection but were variable and undetectable in some animals at later time points (Fig. 1B). Thus, *S. hispidus* appears to be much more resistant to aerosol infection with *M. tuberculosis* H37Rv than guinea pigs infected under identical conditions [15].

Next, we assessed the ability of a much higher challenge dose, 10^8 CFU/ml in the nebulizer, to produce pulmonary and extrapulmonary disease in the cotton rat, as well as the ability of BCG vaccination to protect against this high dose following aerosol challenge. The higher challenge dose resulted in the inhalation and implantation of an average of 272 ± 18 CFU/lung (mean of 10 animals $\pm$ SEM), as determined by plating homogenates of the lungs of animals necropsied one day post-infection. In addition, all cotton rats were productively infected in the lung and the spleen using this higher challenge dose (Fig. 2) as opposed to lower challenge doses (Fig. 1). Figure 2A shows that the bacillary loads in the lungs of non-

vaccinated cotton rats infected with the higher dose of H37RV *Mtb* peaked at 6 weeks post-infection with ~6.5 log CFU and declined thereafter. Prior BCG vaccination protected these animals as bacillary loads were lower at all time intervals. At 6 and 8 weeks post-infection, BCG vaccination reduced lung bacillary loads significantly by 1.65-1.72 log₁₀ (Fig. 2A). In the spleen, bacillary accumulation in the non-vaccinated cotton rats peaked at 4 weeks post-infection and slowly declined thereafter (Fig. 2B). BCG vaccination significantly reduced splenic bacillary loads by 0.83-1.46 log₁₀ at 4 and 6 weeks post-infection compared to the non-vaccinated group. Very few mycobacteria were recovered from the spleens of BCG-vaccinated cotton rats, and none were detected in the nonvaccinated cotton rats at 2 weeks post-infection, indicating that extrapulmonary dissemination of *Mtb* to the spleen may occur at about the same interval following aerosol challenge as has been observed in guinea pigs [15]. In order to protect, BCG must disseminate from the injection site and replicate in lymphoid and other organs. In order to demonstrate that dissemination and persistence of the vaccine had occurred, low levels of viable *M. bovis* BCG were cultured from the injection site, draining lymph nodes and spleens of vaccinated cotton rats not challenged with virulent *M. tuberculosis* up to 10 weeks after subcutaneous vaccination (Fig. 3).

Histological evaluation of granulomas

Figure 4 illustrates H&E-stained sections of pulmonary granulomas from BCG-vaccinated (A) and non-vaccinated (B) cotton rats at 8 weeks post-infection with virulent *M. tuberculosis*. Histological examination of lung lesions from non-vaccinated cotton rats revealed the presence of numerous, loosely organized granulomas consisting principally of epithelioid macrophages, with a peripheral accumulation of lymphocytes (Fig. 4B). Pulmonary granulomas from BCG-vaccinated cotton rats were smaller and more organized with increased mononuclear cellularity and clusters of fibrous debris (Fig. 4A). Notably absent from the lesions of non-vaccinated cotton rats were giant cells and necrosis. Caseous necrosis would be observed routinely in the granulomas of guinea pigs infected under these conditions [16].

Delayed hypersensitivity skin reactions

Following the intradermal injection of PPD (5 γ g) at 8 weeks post-infection, *Mtb*-infected cotton rats were devoid of any dermal inflammation in the dorsal skin. No visible reactions were observed at the injection site 24, 48 and 72 hours following administration of the test in either BCG-vaccinated or nonvaccinated, *Mtb*-infected animals (data not shown). Despite bacteriological and histopathological evidence of extensive mycobacterial infection in these animals (Fig. 2 & 4), we did not observe a positive PPD skin test response in any cotton rat tested as assessed by visible erythema and/or induration. This is the same batch of PPD which we use routinely to induce strong dermal reactions in *Mtb*-infected guinea pigs, proving that the PPD itself was perfectly capable of inducing a DTH response.

In vitro antigen-induced T-Lymphocyte responses

In order to assess the ability of the BCG vaccine to induce mycobacterial-specific, T cell-mediated immunity, PPD-induced lymphoproliferation *in vitro* was evaluated in the splenocyte cultures of BCG-vaccinated, non-infected cotton rats as well as BCG-vaccinated and non-vaccinated, *Mtb*-infected animals. The data in Figure 5A demonstrate that whole splenocytes isolated from BCG-vaccinated, uninfected cotton rats 8-10 weeks post-vaccination proliferated strongly in response to stimulation with the non-specific T cell mitogen, ConA, and to a lesser extent with PPD. Six weeks following virulent challenge, splenocyte proliferation in response to both ConA and PPD was notably reduced in BCG-vaccinated, *Mtb*-infected cotton rats compared with non-vaccinated, *Mtb*-infected animals (data not shown). These data provide evidence that antigen-specific T-cell-mediated immunity can be induced by BCG vaccination in the cotton rat.

To further determine whether BCG vaccination induced a relevant T-cell-mediated immune response in *Mtb*-infected cotton rats, we measured antigen-induced IFN γ protein production. Figure 5B illustrates the concentrations of IFN γ protein released into the supernatant fluids of splenocytes from *Mtb*-infected cotton rats following 24 h stimulation with either ConA or PPD. PPD-induced IFN γ secretion was significantly upregulated in splenocytes obtained from BCG-vaccinated, *Mtb* infected cotton rats compared to the non-vaccinated group. Con A also induced a significant IFN γ response in the BCG-vaccinated group. These data show that BCG vaccination induces a mycobacterial antigen-specific cytokine response which is thought to be associated with vaccine-induced protection [14, 17].

Discussion

Eventual control of TB will depend upon the application of new knowledge and new tools which will be developed using biologically relevant animal models. Indeed, experimental animals have been used to evaluate the mechanisms responsible for natural as well as vaccine-induced immunity against TB; and to test new vaccine candidates needed to augment the current BCG vaccine [18-21]. The mouse and guinea pig have played dominant roles in these efforts [22], although each of these models suffers from disadvantages. Most mouse strains do not develop human-like disease, while guinea pigs lack biological reagents for immunological studies. The American cotton rat may represent a novel animal model of TB incorporating the best features of the current models. Indeed, cotton rats have proven useful for the study of human respiratory viral infections in humans, i.e., respiratory syncytial virus (RSV), influenza virus and parainfluenza virus [23-25]. The cotton rat has several advantages, including their small size, inbred status, and numerous commercial reagents available with which to study immune response mechanisms and disease pathogenesis.

In 2007, Elwood et al performed the first studies of TB since 1940 using the cotton rat [10]. They concluded that cotton rats were susceptible to both aerosol and intranasal infection using relatively low doses of *M. tuberculosis* H37Rv and that the pathology observed in this animal model closely resembled the histopathological changes found during pulmonary infection in humans. Moreover, this group recently concluded that *S. hispidus* may be a good model to study latent TB infection as positive lung cultures for *Mtb* were observed without histologic evidence of disease [11]. Accordingly, our data demonstrate the ability of the cotton rat to sustain relatively constant, moderate levels of viable mycobacteria over 8 weeks following a low challenge dose without evidence of overt disease (Fig. 1). Elwood et al postulated that their findings are consistent with the theory that bacteria are constantly disseminating and reactivating at a low level but are kept in check by the hosts' immune system. However, analysis involving immunomodulators such as TNF inhibitors and steroids will be necessary to further development of this model.

Both studies published by this group show great variability within groups and no statistical analyses were performed in any of their studies. For example, a third of their cotton rats inoculated intranasally with *Mtb* died prior to their 9 month time point; only two thirds of the animals showed histologic evidence of active pulmonary tuberculosis; and only half of the animals were susceptible to immunosuppression with cyclophosphamide at 9 months post-infection [11]. This is consistent with our data which demonstrate that cotton rats infected by the aerosol route at the lower nebulizer concentrations (10^6 and 10^7 CFU/ml) showed great variability in recoverable bacillary loads. Elwood and colleagues postulated that this variability could be attributed to the route of infection and the bacterial strain of *Mtb* used. However, this trend was seen in our experiments using the same mycobacterial strain (H37Rv) which is highly virulent in guinea pigs infected under identical conditions [26], so there must be another reason for the high level of between-animal variability.

Our data demonstrate that *S. hispidus* is much more resistant to infection by the pulmonary route with *Mtb* H37Rv than guinea pigs. Cotton rats failed to develop classical pulmonary granulomas with central necrosis, and failed to express a DTH response following intradermal injection of PPD in spite of evidence of extensive disease 8 weeks post-infection. These observations are in sharp contrast to earlier reports of more tightly organized, necrotic pulmonary granulomas and a measurable DTH response following intradermal injection of PPD [10]. These discrepancies between the studies of Elwood, et al. and ours likely cannot be attributed to strain or dose of *Mtb* and the route of infection used. Likewise, the absence of a DTH reaction in our study is not likely due to differences in PPD source or concentration, but may be attributed to the method of application and assessment of the reaction. Elwood et al. did not confirm their DTH reaction histologically [10].

We demonstrate here for the first time that cotton rats are protected significantly from pulmonary *Mtb* infection by BCG vaccination. We cultured low levels of BCG from the injection site, draining lymph nodes, and spleen up to 10 weeks post-vaccination, indicating dissemination and active replication of BCG in cotton rats. BCG vaccination prior to virulent infection significantly reduced bacillary loads in the lungs and spleens of the cotton rats by 1-1.5 log. This level of protection is comparable to that seen in BCG-vaccinated mice infected under similar circumstances [27,28]. In guinea pigs, BCG vaccination normally induces a greater degree of protection, especially in the spleen [29]. Moreover, vaccination in cotton rats induced an antigen-specific T-cell mediated immune response as measured by enhanced PPD-induced IFN γ production 6 weeks post-infection.

The failure of cotton rats to develop any visible DTH reactions despite the obvious bacteriological and histopathological evidence of disease directly contradicts the previous report [10]. The lack of antigen specific skin test reactivity did not reflect the absence of PPD-responsive T cells in our cotton rats, since splenocytes from *Mtb*-infected cotton rats proliferated and produced IFN γ following stimulation with PPD *in vitro*. Thus, the cotton rat is similar to the mouse in that intradermal PPD skin testing cannot be used to demonstrate the presence of a mycobacterial-specific T-cell response. In mice, the footpad test is used to replace the intradermal skin test [30]. We did not attempt to evaluate the usefulness of the footpad test in cotton rats.

Taken together, our results support the conclusion that pulmonary TB infection in the cotton rat is much more like the course of disease in the mouse than in guinea pigs infected under similar conditions. Relative resistance to pulmonary infection, the ability of the animal to sustain high (and unchanged) bacillary loads in the lungs over several weeks, the lack of necrotic granulomas, and the failure to respond to an intradermal PPD skin test are all well-established features of the mouse model of TB [22,27]. On the other hand, BCG did afford significant protection and induced a T-cell proliferative and IFN γ response to PPD *in vitro*. The degree of vaccine-induced protection, as measured by reductions in lung and spleen bacillary loads, was similar in magnitude to that observed in mice compared to guinea pigs. The apparent advantages of the cotton rat, namely, small size and reagent availability, are also shared with the mouse model. Given these extensive similarities, our results suggest that the cotton rat does not offer any advantages over the mouse model of pulmonary TB in terms of testing novel TB vaccine candidates.

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References

1. McMurray DN. Determinants of vaccine-induced resistance in animal models of pulmonary tuberculosis. *Scand J Infect Dis* 2001;33(3):175–8. [PubMed: 11303805]
2. Orme IM. Mouse and guinea pig models for testing new tuberculosis vaccines. *Tuberculosis (Edinb)* Jan-Mar;2005 85(1-2):13–7. [PubMed: 15687022]
3. Basaraba RJ. Experimental tuberculosis: the role of comparative pathology in the discovery of improved tuberculosis treatment strategies. *Tuberculosis (Edinb)* Aug;2008 88(Suppl 1):S35–47. [PubMed: 18762152]
4. Jain SK, Hernandez-Abanto SM, Cheng QJ, Singh P, Ly LH, Klinkenberg LG, et al. Accelerated detection of *Mycobacterium tuberculosis* genes essential for bacterial survival in guinea pigs, compared with mice. *J Infect Dis* Jun 1;2007 195(11):1634–42. [PubMed: 17471433]
5. Niewiesk S, Prince G. Diversifying animal models: the use of hispid cotton rats (*Sigmodon hispidus*) in infectious diseases. *Lab Anim* Oct;2002 36(4):357–72. [PubMed: 12396279]
6. Boukhvalova MS, Prince GA, Blanco JC. Respiratory syncytial virus infects and abortively replicates in the lungs in spite of preexisting immunity. *J Virol* Sep;2007 81(17):9443–50. [PubMed: 17596309]
7. Yim KC, Carroll CJ, Tuyama A, Cheshenko N, Carlucci MJ, Porter DD, et al. The cotton rat provides a novel model to study genital herpes infection and to evaluate preventive strategies. *J Virol* Dec;2005 79(23):14632–9. [PubMed: 16282463]
8. Richter BW, Onuska JM, Niewiesk S, Prince GA, Eichelberger MC. Antigen-dependent proliferation and cytokine induction in respiratory syncytial virus-infected cotton rats reflect the presence of effector-memory T cells. *Virology* Jun 20;2005 337(1):102–10. [PubMed: 15914224]
9. Blanco JC, Pletneva L, Boukhvalova M, Richardson JY, Harris KA, Prince GA. The cotton rat: an underutilized animal model for human infectious diseases can now be exploited using specific reagents to cytokines, chemokines, and interferons. *J Interferon Cytokine Res* Jan;2004 24(1):21–8. [PubMed: 14980081]
10. Elwood RL, Wilson S, Blanco JC, Yim K, Pletneva L, Nikonenko B, et al. The American cotton rat: a novel model for pulmonary tuberculosis. *Tuberculosis (Edinb)* Mar;2007 87(2):145–54. [PubMed: 16973421]
11. Elwood RL, Rajnik M, Wilson S, Yim K, Blanco JC, Nikonenko B, et al. Characterization of late tuberculosis infection in *Sigmodon hispidus*. *Tuberculosis (Edinb)* Mar;2009 89(2):183–8. [PubMed: 19223233]
12. Grover AA, Kim HK, Wiegshauss EH, Smith DW. Host-parasite relationships in experimental airborne tuberculosis. II. Reproducible infection by means of an inoculum preserved at –70 C. *J Bacteriol* Oct;1967 94(4):832–5. [PubMed: 4964472]
13. Lasco TM, Yamamoto T, Yoshimura T, Allen SS, Cassone L, McMurray DN. Effect of *Mycobacterium bovis* BCG vaccination on *Mycobacterium*-specific cellular proliferation and tumor necrosis factor alpha production from distinct guinea pig leukocyte populations. *Infect Immun* Dec;2003 71(12):7035–42. [PubMed: 14638793]
14. Yamamoto T, Lasco TM, Uchida K, Goto Y, Jeevan A, McFarland C, et al. *Mycobacterium bovis* BCG vaccination modulates TNF-alpha production after pulmonary challenge with virulent *Mycobacterium tuberculosis* in guinea pigs. *Tuberculosis (Edinb)* Mar;2007 87(2):155–65. [PubMed: 17289434]
15. Smith DW, McMurray DN, Wiegshauss EH, Grover AA, Harding GE. Host-parasite relationships in experimental airborne tuberculosis. IV. Early events in the course of infection in vaccinated and nonvaccinated guinea pigs. *Am Rev Respir Dis* Dec;1970 102(6):937–49. [PubMed: 4991996]
16. Lasco TM, Cassone L, Kamohara H, Yoshimura T, McMurray DN. Evaluating the role of tumor necrosis factor-alpha in experimental pulmonary tuberculosis in the guinea pig. *Tuberculosis (Edinb)* Jul;2005 85(4):245–58. [PubMed: 15958260]
17. Ly LH, Russell MI, McMurray DN. Microdissection of the cytokine milieu of pulmonary granulomas from tuberculous guinea pigs. *Cell Microbiol* May;2007 9(5):1127–36. [PubMed: 17217432]

18. Ly LH, McMurray DN. Tuberculosis: vaccines in the pipeline. *Expert Rev Vaccines* Jul;2008 7(5): 635–50. [PubMed: 18564018]
19. McMurray DN. A coordinated strategy for evaluating new vaccines for human and animal tuberculosis. *Tuberculosis (Edinb)* 2001;81(1-2):141–6. [PubMed: 11463235]
20. Orme IM. Potential complications to TB vaccine testing in animal models. *Indian J Exp Biol* Jun; 2009 47(6):440–4. [PubMed: 19634708]
21. Dharmadhikari AS, Nardell EA. What animal models teach humans about tuberculosis. *Am J Respir Cell Mol Biol* Nov;2008 39(5):503–8. [PubMed: 18556589]
22. McMurray DN. Disease model: pulmonary tuberculosis. *Trends Mol Med* Mar;2001 7(3):135–7. [PubMed: 11286786]
23. Ottolini MG, Blanco JC, Eichelberger MC, Porter DD, Pletneva L, Richardson JY, et al. The cotton rat provides a useful small-animal model for the study of influenza virus pathogenesis. *J Gen Virol* Oct;2005 86(Pt 10):2823–30. [PubMed: 16186238]
24. Ottolini MG, Porter DD, Blanco JC, Prince GA. A cotton rat model of human parainfluenza 3 laryngotracheitis: virus growth, pathology, and therapy. *J Infect Dis* Dec 15;2002 186(12):1713–7. [PubMed: 12447755]
25. Prince GA, Horswood RL, Camargo E, Koenig D, Chanock RM. Mechanisms of immunity to respiratory syncytial virus in cotton rats. *Infect Immun* Oct;1983 42(1):81–7. [PubMed: 6352505]
26. Converse PJ, Eisenach KD, Theus SA, Nuermberger EL, Tyagi S, Ly LH, Geiman DE, Guo H, Nolan ST, Akar NC, Klinkenberg LG, Gupta R, Karakousis PC, Lamichhane G, Lun S, McMurray DN, Grosset JH, Bishai WR. The impact of mouse passaging on *Mycobacterium tuberculosis* strains prior to virulence testing in the mouse and guinea pig aerosol models. *Infect Immun*. 2009 submitted.
27. Orme IM. The mouse as a useful model of tuberculosis. *Tuberculosis (Edinb)* 2003;83(1-3):112–5. [PubMed: 12758199]
28. Baldwin SL, D'Souza C, Roberts AD, Kelly BP, Frank AA, Lui MA, et al. Evaluation of new vaccines in the mouse and guinea pig model of tuberculosis. *Infect Immun* Jun;1998 66(6):2951–9. [PubMed: 9596772]
29. Wiegshaues EH, McMurray DN, Grover AA, Harding GE, Smith DW. Host-parasite relationships in experimental airborne tuberculosis. 3. Relevance of microbial enumeration to acquired resistance in guinea pigs. *Am Rev Respir Dis* Sep;1970 102(3):422–9. [PubMed: 5450906]
30. Kubica GP, Dunbar FP, Kim TH. Response of hypersensitive mice to the footpad injection of living homologous or heterologous mycobacteria: preliminary report. *Appl Microbiol* May;1973 25(5):718–23. [PubMed: 4577173]

A.

B

Figure 1.

The effect of aerosol challenge dose in cotton rats (*S. hispidus*) on progression of mycobacterial infection in the lung (closed bars) and spleen (open bars). Bars represent Mean Log₁₀ CFU ± SEM for 4-5 animals at 2, 4, 6, and 8 weeks post infection with *M. tuberculosis* H37Rv. A. Nebulizer concentration = 10⁶ CFU/ml. B. Nebulizer concentration = 10⁷ CFU/ml. Minimum limits of detection in the lung = 1.35 log₁₀ and in the spleen = 1.0 log₁₀. Values in parentheses indicate the proportion of animals in which bacteria were detected.

Figure 2.

The effect of BCG vaccination on mycobacterial infection in the (A) lung and (B) spleen at two week intervals post- infection with a nebulizer concentration of 10^8 CFU/ml of *M. tuberculosis* H37Rv. Bars represent Mean Log₁₀ CFU $\pm$ SEM for 4-5 animals. Asterisks denote a statistically significant difference between BCG-vaccinated and non-vaccinated animals, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

Figure 3. BCG vaccine organisms cultured from the injection site, draining lymph nodes (DLN) and spleens of BCG-vaccinated cotton rats. Animals were injected subcutaneously with 10^4 CFU of BCG (Statens Serum Institut) and rested for 10 weeks prior to aseptic removal of each tissue. Bars represent Mean Log₁₀ CFU $\pm$ SEM for 4-5 animals.

Figure 4. Representative H&E stained lung granulomas in BCG-vaccinated (A) and non-vaccinated (B) cotton rats. Eight weeks post-challenge with virulent *M. tuberculosis* H37Rv by the respiratory route, lung lobes were aseptically removed and fixed in 10% formalin prior to sectioning and staining.

Figure 5.

Cotton rat splenocyte proliferative response to Con A (5.0 $\mu\text{g/ml}$) and PPD (12.5 $\mu\text{g/ml}$). Panel A illustrates the proliferative response in BCG vaccinated, uninfected cotton rats, 8-10 weeks post BCG vaccination. Panel B illustrates IFN γ protein levels (pg/ml) in the supernatant fluids of non-vaccinated (closed bars) and BCG-vaccinated (open bars) cotton rat splenocytes stimulated with either ConA (5.0 $\mu\text{g/ml}$) or PPD (12.5 $\mu\text{g/ml}$) at 6 weeks post aerosol challenge with *Mtb*. Results shown are Mean IFN γ protein concentrations $\pm$ SEM (n=6 cotton rats) following 24 hrs stimulation.