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Assessment of vaccine testing at three laboratories using the guinea pig model of tuberculosis

Ajay Grover¹, JoLynn Troudt¹, Kimberly Arnett¹, Linda Izzo¹, Megan Lucas¹, Katie Strain¹, Christine McFarland², Yper Hall³, David McMurray², Ann Williams³, Karen Dobos¹, and Angelo Izzo^{1,¶}

¹Colorado State University, Department of Microbiology, Immunology & Pathology, Fort Collins, CO, USA

²Department of Microbial and Molecular Pathogenesis, College of Medicine, Texas A&M Health Science Center, College Station, TX, USA

³Health Protection Agency, Microbiological Services, Porton Down Salisbury, UK

Abstract

The guinea pig model of tuberculosis is used extensively in different locations to assess the efficacy of novel tuberculosis vaccines during pre-clinical development. Two key assays are used to measure protection against virulent challenge: a 30 day post-infection assessment of mycobacterial burden and long term post-infection survival and pathology analysis. To determine the consistency and robustness of the guinea pig model for testing vaccines, a comparative assessment between three sites that are currently involved in testing tuberculosis vaccines from external providers was performed. Each site was asked to test two “subunit” type vaccines in their routine animal model as if testing vaccines from a provider. All sites performed a 30 day study, and one site also performed a long-term survival/pathology study. Despite some differences in experimental approach between the sites, such as the origin of the *M. tuberculosis* strain and the type of aerosol exposure device used to infect the animals and the source of the guinea pigs, the data obtained between sites were consistent in regard to the ability of each “vaccine” tested to reduce the mycobacterial burden. The observations also showed that there was good concurrence between the results of short-term and long-term studies. This validation exercise means that efficacy data can be compared between sites.

Keywords

tuberculosis; vaccines; guinea pig

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[¶]Corresponding author: Mailing address: Colorado State University, Department of Microbiology, Immunology & Pathology, 1682 Campus Delivery, Fort Collins CO 80523 Phone: (970) 491-4350. Fax: (970) 491-1815. Angelo.Izzo@colostate.edu.

Conflict of interest

The authors have no conflict of interest.

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Introduction

Infection with *Mycobacterium tuberculosis* remains a world-wide public health problem, which has been made even more urgent by the emergence of multi- and extensively-drug resistant strains. The need to develop new vaccines to prevent the progression of this pandemic is evident from the growth in the number of new vaccines that are currently in the pipeline, with 11 vaccines currently in various stages of clinical trials.¹ Prior to novel tuberculosis vaccine candidates reaching the clinical trials stage, it has been customary that they undergo a series of tests involving animal models to determine several factors such as their ability to induce Th1 immunity and to reduce the mycobacterial burden after infection with virulent *M. tuberculosis*.² The small animal models of tuberculosis that are routinely used to assess vaccines include the mouse and the guinea pig. The mouse model provides an expeditious means of determining whether a candidate can induce an immune response capable of reducing the mycobacterial burden after a low dose aerosol with virulent *M. tuberculosis*.² The immune response generated by the vaccine before and after infection can be monitored. Thus from the mouse model, one can determine the phenotype of the immune response generated and whether it induced a memory T cell response. In mice, *M. bovis* BCG vaccination is used as a positive control, to ensure that the experiment is functioning optimally and typically provides approximately 1Log₁₀ reduction in colony forming units (CFU) in the lung and spleen.³ The guinea pig model of tuberculosis has been used extensively to test novel vaccines and has aided in identifying candidates that are currently in clinical trials.⁴⁻⁶ The guinea pig is highly susceptible to infection with low numbers of *M. tuberculosis* and develops disease that is observed in humans and eventually succumbs to infection as a result of weight loss and decreased pulmonary function due to extensive pulmonary infiltration. The two commonly used forms of the model include a short-term, defined-time-point study that is similar to the short-term mouse model, in which guinea pigs are vaccinated, infected with a low dose aerosol of *M. tuberculosis* and the CFU determined at 4–5 weeks post-infection.³ The second, is a long-term survival study, which involves vaccinating and infecting guinea pigs, monitoring them for disease symptoms and then euthanizing the animals as they begin to succumb to disease symptoms.⁷ The long-term studies commonly involve assessment of pathology at the time of necropsy to determine the extent of lung involvement and the types of granulomas formed. A vaccine is expected to prevent or significantly delay weight loss and limit pulmonary pathology and thus prolong survival of an infected guinea pig. The guinea pig model has represented a critical link between pre-clinical testing and human trials in the pipeline of tuberculosis vaccine candidates and a better understanding of how the model fits into the progression of vaccines is required. Although there are a limited number of reagents currently available to investigate immune responses in guinea pigs, several studies have been published to indicate that this trend is changing.^{7,8} In addition to critical information such as pathology and bacterial load, more immune studies are now possible in the guinea pig, with the potential that the same immune assays can be performed in the mouse, guinea pig and eventually humans leading to a backwards evaluation of vaccines and continuous improvements in the interpretation of the models.

Early reports documented the fact that when different animal models were used, with different readouts (e.g. survival, lung score and colony forming units) to measure response to infection, the same panel of tuberculosis vaccines was ranked in widely different order, both between laboratories⁹ and within the same laboratory.¹⁰ However, there is a paucity of published data on the uniformity of results obtained by testing the same panel of vaccines in essentially the same animal model in different laboratories. Since novel tuberculosis vaccines are currently being tested in guinea pigs by several laboratories around the world, it was of interest to determine how comparable the results would be if the same vaccine were evaluated in three separate sites that routinely use the guinea pig model to assess vaccines:

two in the United States of America (CSU and TAMUHSC) and one in the United Kingdom (HPA). To achieve this goal, all three sites received the same vaccines, which were to be used in the routine assay performed for that laboratory. The intent of the study was to compare outcomes from the three sites using the guinea pig models routinely used at each site to assess novel vaccines. Variability in assessing the vaccines was in the way in which each site performed their own particular animal study based on their standard protocols. Overall, and despite some variations in the protocol used in each laboratory, there was a general consensus between all sites in that each vaccine performed in a comparable way relative to positive and negative control groups.

Materials and Methods

Sites

The three sites that participated in the study were Colorado State University (CSU), Fort Collins, Colorado, Texas A&M Health Science Center (TAMHSC), College Station, Texas and the Health Protection Agency (HPA), Porton Down, UK. All sites routinely perform vaccine assessment studies for the tuberculosis vaccine research community using the guinea pig model of tuberculosis.

Protein subunit vaccine preparation

Two “test” vaccines were chosen for distribution, one consisted of CFP (culture filtrate protein) and the other was recombinant antigen 85A (recAg85A), both produced by the NIH Vaccine Testing and Research Material (TBVTRM) contract at Colorado State University (NIH/HHSN266200400091c). CFP was prepared from *M. tuberculosis* H37Rv as described previously¹¹ with the following modifications. Briefly, CFP was dried and non-covalently associated lipids removed by two extractions with chloroform:methanol (2:1), followed by two extractions with chloroform:methanol:water (10:10:3), with 2 hr incubations for each extraction. The pellet was dried under nitrogen, and resuspended in sterile PBS; DNase and RNase were added, and the sample was incubated at 4°C for 1hr. RecAg85A was produced in and purified from *Escherichia coli*. The gene fragment encoding the mature Ag 85A was amplified by PCR using the primers

CCATACTACCTCCATATGTTTTCCCGGCCGGGCTTG (forward) and GCAGTTCGGATCCCTAGGCGCCCTGGGG (reverse). The amplified gene product was ligated into the multiple cloning site for pET15B (EMD Biosciences, Gibbstown, NJ) following digestion with *Nde*I and *Bam*HI and the recombinant plasmid was transformed into *E. coli* BL21(DE3)pLysS (Invitrogen, Carlsbad, CA). The transformed *E. coli* was plated onto LB medium containing ampicillin and chloramphenicol, followed by sequential inoculation of 30 ml, 150 ml, and finally 5L of HyperBroth (Athena Enzyme Systems, Baltimore MD) and cultures grown to 1.35 OD₆₀₀ using a BioFlow 110 Modular Benchtop Fermentor (New Brunswick Scientific, Edison, NJ). The cultures were induced with 0.5 mM IPTG and harvested after 18 hrs of growth at 25°C. The cells were broken by probe sonication and soluble recAg85A was purified by our standard methods as described previously.¹² Both recAg85A and CFP were tested for endotoxin (*Limulus amoebocyte* lysate QCL-1000 kit, Cambrex, Walkersville, MD) and contained less than 10 ng EU/mg of protein.

Monophosphoryl Lipid A (MPL), a detoxified derivative of lipid A from Gram-negative bacteria and dimethyl dioctadecyl ammonium bromide (DDA) (Sigma) were purchased and sent to each site with the test vaccines so as to ensure each site used the same lots. At each site, proteins were emulsified in (DDA) with MPL. MPL (Sigma) was prepared as previously described¹³ in 0.5% (v/v) triethanolamine in water, dispersed using repeated cycles of heating (65°C) and sonication. MPL, DDA and protein were combined

immediately prior to use, heated to 70–80°C for 30 seconds and then sonicated for 30 seconds. Heating and sonication were repeated 3 times in total.

Mycobacteria

CSU—*M. bovis* BCG, Pasteur strain (TMC#1011,) was grown in Proskaur and Beck (P&B) medium with 0.1% Tween80 to mid-log phase. Aliquots were then stored at –80°C and thawed when used. *M. tuberculosis*, H37Rv (TMC#102) was initially grown as a pellicle on P&B medium for three passages and then expanded to produce working stocks in P&B medium with 0.1% Tween. At the mid-log phase of growth aliquots were prepared and stored at –80°C.

HPA—BCG vaccine (Danish 1331, Statens Serum Institut, Copenhagen, DK) was reconstituted just prior to use in Sauton diluent provided by the manufacture. For administration at the required dose, the vaccine was further diluted in sterile PBS. *M. tuberculosis* H37Rv (NCTC 7416) challenge suspension was generated from frozen aliquots (–80°C) of high titre chemostat culture grown to steady-state conditions as previously described.¹⁴

TAMHSC—BCG vaccine (Danish 1331; Statens Serum Institut, Copenhagen, DK) was used as a positive control. The vaccine was obtained as a lyophilized commercial preparation and reconstituted just prior to use with the diluents provided by the manufacturer. *M. tuberculosis* H37Rv (ATCC 25495) was used to infect the guinea pigs. A frozen stock, stored at –80°C, was prepared using a previously published protocol.¹⁵ An aliquot of the frozen stock was thawed rapidly just prior to use and diluted appropriately for use in the Madison Aerosol Exposure Chamber (University of Wisconsin Engineering Shops, Madison, WI) according to published protocols.¹⁰

Animals and Ethics statement

CSU—Outbred female Hartley guinea pigs weighing 450–500 grams were purchased from Charles River Laboratories (Wilmington, MA). Guinea pigs were maintained under barrier conditions in isolator cages (Thoren, Hazleton, PA) for the entire period of the experiment, during which time they had access to chow and water *ad libitum* and daily enrichment. All experimental procedures at CSU were approved by the Animal Care and Use Committee.

HPA—Full-barrier reared out-bred female Dunkin-Hartley guinea pigs, 300 – 350 gm, were purchased from the UK Home Office approved breeder and supplier Harlan, UK, and maintained in cages in groups of 6 to 8 within an ACDP biocontainment level 2 HEPA-filtered room, with *ad libitum* access to food and sterile water. After challenge, animals were housed in pairs at ACDP biocontainment Level 3 within HEPA filtered negative pressure isolators, with *ad libitum* access to food and sterile water. All animal work at HPA was subject to local ethical review and was conducted by personal licence holders under a project licence approved by the UK Home Office. The housing and environmental conditions for all animal work were fully compliant with UK Home Office “Code of Practice for the Housing and Care of Animals Used in Scientific Procedures, 1989” and appropriate enrichment was provided throughout the experimental period.

TAMHSC—Male and female, random-bred, Hartley strain guinea pigs were purchased from Charles River, Wilmington, MA and housed individually in polycarbonate cages with micro-isolator lids. Animals received commercial guinea pig chow and tap water supplemented with vitamin C (0.05%) *ad libitum*. They were maintained on a 12/12 hr light/dark cycle in a temperature and humidity controlled environment. Virulent infection and subsequent maintenance of infected animals took place in a certified ABSL-3 facility. The experimental

protocols were approved by the Institutional Animal Care and Use Committee of Texas A&M University.

Animal immunisations

At each site, guinea pigs were immunised 3 times, at 3 week intervals, with 20 µg protein emulsified in DDA (500 µg/dose) and MPL (50 µg/dose), or DDA/MPL only. Protein in adjuvant and adjuvant only were delivered subcutaneously each in 250 µl in the nape of the neck. A control group of guinea pigs received pyrogen-free sterile saline. At CSU, one group received 10^3 CFU of BCG Pasteur via the intradermal route at the time of the last subunit vaccination. Five animals per group were used for the 5 week short-term CFU study and 10 animals per group were used for the long-term survival study. At HPA, at the time of the first subunit vaccination, one group received BCG Danish (SSI) at 5×10^4 CFU, delivered subcutaneously in 250 µl in the nape of the neck. One group remained unvaccinated. Eight animals per group were used. At TAMHSC, one group received 10^3 CFU of BCG Copenhagen in 0.1ml sterile saline subcutaneously in the inguinal region. The placebo group received an identical injection of sterile saline.

Aerosol challenge and determination of bacterial load

CSU—At ten weeks after the final vaccination, guinea pigs were infected with a low dose aerosol using the Madison Aerosol Exposure Chamber (Madison WI). Guinea pigs were infected with 10–20 CFU of virulent *M. tuberculosis* H37Rv. Briefly, bacterial stocks were diluted in 10 ml of sterile distilled water to 1×10^6 CFU/ml and placed in the nebulizer and the animals exposed to the aerosol for 5 min. At 5 weeks post-infection, bacterial load was determined by humanely euthanizing 5 animals per group and plating 10-fold dilutions of homogenates of the right cranial lobe of the lung and half of the spleen onto nutrient 7H11 agar supplemented with OADC. Colonies were enumerated after 21-day incubation at 37°C. The limit of detection for determining bacterial loads was 2 Log₁₀ CFU for the lung and spleen. For survival studies guinea pigs were monitored daily and weighed once each week and guinea pigs were euthanized when they reached set criteria established by the animal care and use committee, such as being moribund or exceeding acceptable weight loss and/or being affected in their respiratory rate (labored/heavy breathing). Time to euthanasia was used as time to death.

HPA—Twelve weeks after the initial immunisation, guinea pigs were challenged with aerosolised *M. tuberculosis* H37Rv (NCTC 7416) using a fully contained, nose-only exposure Henderson apparatus. Aerosol particles with a diameter of 0.5–7.0 µm (mean 2.0 µm) were generated using a 3-jet Collison nebuliser (BGI Inc., USA) from a suspension of H37Rv diluted in sterile distilled water at 3×10^6 /CFUml (verified by subsequent enumeration by plating on 7H11 agar) to give an inhaled retained dose of approximately 20 CFU. Five weeks after challenge, animals were euthanized by intraperitoneal injection of sodium pentobarbital (Dolethal, Vetoquinol UK Ltd) and lungs and spleen were removed aseptically. The spleen minus a small apical section and the left apical, cardiac, right cardiac and right diaphragmatic lung lobes were homogenised in 5 and 10 ml sterile water, respectively. Serial dilutions were plated on Middlebrook 7H11 selective agar (bioMerieux UK Ltd), 100 µl per plate in duplicate and incubated at 37°C. After 4 weeks, plates were examined for determination of bacterial load (CFU per tissue sample), calculated by multiplying homogenate cfu/ml by homogenate volume. Where no colonies were observed, a minimum detection limit was set by assigning a count of 0.5 colonies, equating to 5 CFU/ml.

TAMHSC—Twelve weeks after the initial immunization, all guinea pigs were challenged by the respiratory route with virulent *M. tuberculosis* H37Rv (ATCC 25495) using a

Madison Aerosol Exposure Chamber according to published protocols.^{10,16} The challenge suspension was generated in a Colison nebulizer (BGI, Inc., USA) from a frozen aliquot (-80°C) prepared as described previously.¹⁵ The concentration of bacteria in the nebulizer was adjusted to give an inhaled retained dose of approximately 20 CFU, based upon many previous experiments. Five weeks after challenge, animals were euthanized by an overdose of sodium pentobarbital (Sleepaway, Fort Dodge Animal Health) injected intraperitoneally. The lungs and spleen were removed aseptically from each guinea pig, and the right lower lung lobe and one-half of the spleen were homogenized separately in 5 ml sterile physiological saline. Serial, 10-fold dilutions were inoculated in duplicate onto Middlebrook 7H11 agar (BVA Scientific) and incubated at 37°C .¹⁷ After 3–4 weeks, colonies were counted and the mean CFU per pair of plates at the dilution which exhibited the largest countable number was used to calculate the bacterial load (CFU per tissue sample) which was then transformed to $\log_{10}$ before being expressed as mean $\pm$ standard error of the mean (SEM). The minimal detectable level in either tissue using this culture procedure was $\log_{10}$ 1.35, and that value was entered in the data set for all tissues in which no colonies were observed at the lowest dilution for purposes of statistical analysis.

Histology

The right caudal lobe of the guinea pig lung was utilized to analyze pathological lesions. The excised lobe was inflated with formalin and placed in total into formalin. For processing the lobe was embedded in paraffin and sections cut and stained with Hematoxylin and Eosin (H&E; IHC Tech, Aurora, CO). Photomicrographs were taken using an Olympus BX41 microscope attached to a Dell Precision computer with DP2-BSW software for image capture.

Statistical Analysis

Each site was encouraged to use their standard protocol for determining statistical significance between treatment groups. At CSU and TAMHSC a one Way Analysis of Variance of $\log_{10}$ -transformed CFU with an all pair-wise multiple comparison procedure (Bonn Bonferroni t-test) was used to compare data from the different sites. At HPA pair-wise analysis of groups was performed using the Mann-Whitney non-parametric test. Guinea pig survival was plotted using the Kaplan-Meier method, and differences between curves were analyzed using the log-rank test.

Results

Short-term guinea pig model

When all the vaccine formulations were tested in the short-term guinea pig model, there was good agreement between all 3 sites in regard to determining which formulation was able to significantly reduce the mycobacterial burden in the lungs and spleens. Firstly, when considering just the CFU at 5 weeks post-infection in the lungs of saline-treated groups as an indicator of infection status of guinea pigs (Figure 1), there was similarity between CSU and HPA, while at TAMHSC, a lower bacterial burden was observed. BCG is used as a positive control to ensure the reliability of the experiment and usually induces a significant reduction in CFU when compared to the saline-treated or unvaccinated control group. When guinea pigs were vaccinated with BCG prior to infection, the reduction in the number of CFU obtained in the lungs was similar between sites ($\text{CSU} = 1.47\log_{10}\text{CFU}$, $\text{HPA} = 1.35\log_{10}\text{CFU}$ and $\text{TAMUHSC} = 1.72\log_{10}\text{CFU}$), all of which were statistically significant ($\text{CSU} \& \text{TAMUHSC } p < 0.001$, $\text{HPA } p < 0.01$).

At all sites, there was a significant reduction in CFU in the lungs of CFP/MPL/DDA inoculated animals ($\text{CSU} \& \text{HPA } p < 0.01$ and $\text{TAMHSC } p < 0.05$) compared to the Saline-

treated/unvaccinated group. For Ag85A inoculated animals, a marginal reduction was observed at all sites, but was statistically significant for the HPA data only ($p < 0.05$) (Figure 1). Analysis of the spleen CFU data (Figure 2) showed a strong agreement between sites in that all sites found significant reductions in CFU for CFP/MPL/DDA inoculated animals when compared to the Saline-treated/unvaccinated group (CSU & TAMHSC $p < 0.05$ and HPA $p < 0.01$), while none found the Ag85A or MPL/DDA control groups to be significantly different. Thus, there was considerable concordance between the three laboratories in terms of the performance of the vaccines in the short-term protocol. That is, BCG- and CFP-vaccinated guinea pigs had significantly fewer CFU in their lungs and spleens than the saline-treated group ($p < 0.001$), while the Ag85A and MPL/DDA control groups did not. These data suggest that each laboratory could reliably distinguish between the vaccine preparations.

Long-term guinea pig model

Prolonged survival and reduced pulmonary pathology after low dose aerosol infection with virulent *M. tuberculosis* has been a hallmark for testing novel vaccines in guinea pigs. Groups inoculated with either BCG or *M. tuberculosis* antigens in MPL/DDA displayed a significantly prolonged survival when compared to saline-treated animals (Figure 3: BCG and CFP $p < 0.001$, Ag85A $p < 0.01$). When compared to MPL/DDA alone, the CFP+MPL/DDA animals survived significantly longer ($p < 0.01$), while the Ag85A+MPL/DDA did not ($p = 0.2$). Survival after vaccination with BCG did not differ significantly from that after vaccination with CFP+MPL/DDA. The MPL/DDA adjuvant-treated control group also had significantly prolonged survival compared to the saline group ($p \leq 0.001$), suggesting an effect of the adjuvant to initiate a strong innate response in a cohort of the guinea pigs. This type of finding is unusual, but does happen and therefore it was important to compare the vaccine formulation treated groups with the adjuvant treated group. Overall the data suggested that inoculation with CFP formulated with MPL/DDA was able to prolong survival and supports the data observed in the short-term model.

Assessment of lung pathology at the time of necropsy revealed the presence of granulomatous lesions in the lungs of all guinea pigs however there were significant differences between groups in the type of lesion. Guinea pigs that were treated with saline or MPL/DDA (Figure 4A and E) had larger lesions that were characterized by the presence of necrotic cores. In BCG-vaccinated animals, lesions (Figure 4B) were smaller and consisted of well-organized accumulation of cells. Of the two protein vaccines, lesions in animals vaccinated with CFP (Figure 4C) resembled those observed in BCG-vaccinated guinea pigs, while those of Ag85A (Figure 4D) vaccinated animals were similar to the saline or MPL/DDA treated animals with notable acellular areas of necrosis visible within lesions.

Discussion

Animal models of tuberculosis have been used extensively to investigate both the pathogen and the host response and have been useful in helping to identify key factors associated with infection.¹⁸ Historically, the guinea pig has been used to model tuberculosis and was used by Koch to establish the etiologic relation of the tubercle bacillus to tuberculosis.¹⁹ Throughout history investigators have relied on the guinea pig to dissect the pathogenesis of tuberculosis and identify factors associated with disease. Indeed, the fact that the guinea pig is very sensitive to infection with *M. tuberculosis* made it, from an early time, an ideal animal with which to diagnose troublesome cases (i.e. sputum negative) of tuberculosis²⁰ and to determine the ability of *M. tuberculosis* to survive in the environment such as in dust material containing living tubercle bacilli.²¹ The guinea pig was also used in early fundamental work to define tuberculin hypersensitivity.²² While the majority of the earlier studies involved the direct inoculation of the organism (usually intradermal or

intramuscular), the advent of aerosol delivery systems has allowed for pulmonary infection of guinea pigs and the ability to study pulmonary pathogenesis of the infection.^{23–25} Toward the latter part of the 20th century, the guinea pig was used by certain groups to identify aspects of the immunopathogenesis of the disease,^{26,27} but the use of the mouse model of tuberculosis was predominant. During this time, the guinea pig model of tuberculosis was also proffered for use as a model to test the ability of novel vaccines to reduce the mycobacterial burden after infection.^{28,29}

The number of novel vaccines against tuberculosis has increased over the past decade, leading to the progression of vaccines into clinical trials.¹ Evaluation of the immunogenicity and the ability to reduce the mycobacterial burden of candidate vaccines in animal models has always been an essential step in the pre-clinical evaluation of new tuberculosis vaccine candidates. Both the mouse and guinea pig models have been used for this purpose, however the guinea pig model is considered a more stringent test of the ability of a vaccine to prevent disease and deaths.^{2,8} The ability of a vaccine candidate to prolong the life of infected guinea pigs has proven to be a reliable parameter. Given the expense and the expertise required to perform these types of studies, the guinea pig model is used in a limited number of locations, which are used by vaccine developers to obtain data on vaccine candidates. Therefore it would be useful to ensure that at a minimum, the key findings from these models are in line with each other, although it must be remembered that there is not a minimum performance requirement in the guinea pig that determines whether a vaccine candidate is allowed to move into the clinic or not. As long as the candidate is either immunogenic for the introduced antigen and shows a positive effect in the model against the defined endpoints, safety and cost will determine its progression into the clinic. So the guinea pig has to be primarily robust, able to see how vaccines are different and not totally misleading, since the model is run in different places using different protocols, knowing that the results are not that different indicates that one can use any of these sites for testing and obtain reliable data. Three laboratories that use the guinea pig model were recruited in this study for the purpose of determining the between-laboratory variability in the assays used to estimate the protective effect of novel vaccine candidates. In the past different models were compared to each other^{9,10} however, this is the first time a comparison of essentially the same model used in different laboratories has been reported. The current data provide important information for vaccine developers, in that it demonstrates the robustness of the model and provides confidence that the guinea pig model can provide similar data for a vaccine candidate regardless of where it is tested, as long as the model is well established at the site. In order to perform these comparative studies, the recombinant proteins and CFP were produced by the NIH TBVTRM contract, the lyophilized proteins were shipped to the sites and each site followed the same protocol for mixing the proteins with adjuvants to prepare subunit vaccines. This is important for the vaccine testing procedures since all sites involved in the vaccine testing follow the vaccine preparation protocol submitted by the vaccine provider. The concordance of the data among the sites confirms the robustness of the guinea pig model as an appropriate means for testing vaccine candidates. All three sites provided data about the vaccines that ranked them in the same order, with most of the parameters that were examined were in fact similar either in quality or quantity of the results. Thus differences in the models, such as the source of guinea pigs, the post-vaccination rest period the type of aerosol exposure device used to infect the animals and the source of the virulent *M. tuberculosis* (Table 1) did not factor into the outcomes obtained from each site supporting the robust nature of the model for testing novel vaccines. In general, there was consensus between the three sites in being able to determine which of the provided vaccines was capable of reducing the mycobacterial burden after low dose aerosol (Figures 1 & 2). The CSU site also performed a long-term survival study (Figures 3 & 4). The results showed that there was good agreement between the results of the short-term and

long-term protocols, and it would be anticipated that testing novel vaccines at these sites will yield similar results regardless of the location.

In the overall scheme of testing novel vaccines against tuberculosis the guinea pig model has proven to be a very useful tool; for almost all of the vaccines under clinical study, progression to clinical development was supported by guinea pig efficacy data. With the development of new reagents to dissect immune responses not only to vaccines but also during infection⁷ the guinea pig model can provide us with a better understanding of disease pathogenesis as well as contribute to the elucidation of the mechanisms of vaccine-induced resistance in tuberculosis. This study demonstrates that the results obtained by different laboratories employing the guinea pig exposure model are truly comparable, allowing a direct comparison of the performance of different vaccines at different sites and avoiding the necessity of retesting the same vaccine at multiple sites.

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Figure 1.

Colony forming units in the lungs of guinea pigs euthanized at 5 weeks post-infection at (A) CSU, (B) HPA and (C) TAMHSC. Bars indicate group median. Animals were inoculated with saline (unvaccinated at HPA), BCG, CFP+MPL/DDA, Ag85A+MPL/DDA or MPL/DDA, rested for 6 or 10 weeks and then infected with a low dose aerosol of virulent *M. tuberculosis* H37Rv. The lungs were excised, homogenized and plated on 7H11 agar. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

Figure 2.

Colony forming units in the spleens of guinea pigs euthanized at 5 weeks post-infection at (A) CSU, (B) HPA and (C) TAMHSC. Bars indicate group median. Animals were inoculated with saline (unvaccinated at HPA), BCG, CFP+MPL/DDA, Ag85A+MPL/DDA or MPL/DDA, rested for 10 weeks and then infected with a low dose aerosol of virulent *M. tuberculosis* H37Rv. The spleens were excised, homogenized and plated on 7H11 agar. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

Figure 3.

Kaplan-Meier plot of the survival of guinea pigs after low dose aerosol infection with virulent *M. tuberculosis* H37Rv. Guinea pigs were inoculated with saline, BCG, CFP+MPL/DDA, Ag85A+MPL/DDA or MPL/DDA and rested for 10 weeks prior to infection. All guinea pigs were monitored after infection for weight loss, increased respiration and decreased activity and euthanized in a humane manner according to the protocols approved by the CSU IACUC. n = 10 guinea pigs per group.

Figure 4. Representative photomicrographs of lung tissue sections from infected guinea pigs at the time of necropsy. Lungs from guinea pigs inoculated with saline (A), BCG (B), CFP+MPL/DDA (C), Ag85A+MPL/DDA (D) or MPL/DDA (E) were fixed in formalin, sectioned and stained with hematoxylin and eosin to examine the nature of the granulomas.

Table 1

Table showing the differences and similarities in parameters used to evaluate “Short-term guinea pig model” at the three sites involved in the study.

Parameters	CSU	HPA	TAMHSC
BCG strain and Immunization	Pasteur -10 ³ CFU via intradermal route at the time of the last subunit vaccination	Danish 1331-5×10 ⁴ CFU via subcutaneous route at the time of first subunit vaccination	Danish 1331 -10 ³ CFU via subcutaneous route at the time of the last subunit vaccination
<i>M. tuberculosis</i> strain	H37Rv (TMC#1011)	H37Rv (NCTC 7416)	H37Rv (ATCC 25495)
Aerosol Challenge	At 10 weeks after the final vaccination using Madison Aerosol Chamber-delivered 10–20 CFU in Lungs	At 12 weeks after the final vaccination using Henderson apparatus- delivered 20 CFU in Lungs	At 12 weeks after the final vaccination using Madison Aerosol Chamber- delivered 20 CFU in Lungs
Bacterial Load Determination	5 weeks post-infection	5 weeks post-infection	5 weeks post-infection
CFU Counting	21 days after plating	30 days after plating	21–30 days after plating