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Therapeutic vaccination against relevant high virulence clinical isolates of *Mycobacterium tuberculosis*

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SUMMARY

The purpose of this study was to attempt to develop therapeutic or post-exposure vaccines that could slow progressive disease in guinea pigs infected by low dose aerosol with very high virulence Beijing isolates of *Mycobacterium tuberculosis*, currently classified as Category C biodefense pathogens by the NIH and CDC. After screening several candidates we focused on the use of three candidates; these were a pool of bacterial iron acquisition proteins, a pool of antigens recognized by T cells from chronically infected mice thought to represent proteins made by the bacillus in response to decreases in local oxygen tension, and a bacterial lipoprotein that is a potent TLR2 agonist. When delivered in a potent GLA-based adjuvant [targeting TLR4 and TLR9], in most cases we were unable to reduce the bacterial load in the lungs. However, the pathologic appearance of lungs from these animals showed that, while primary lesions were most unaffected and had become necrotic, the development of large, lung consolidating secondary lesions seemed to have been mostly prevented. In animals given both a priming vaccination and a boost the effects were prominent, and almost certainly contributed to significantly prolonged survival in these animals. In a biodefense situation, this prolonged survival would be potentially long enough to allow for the organism to be identified and a drug susceptibility profile determined.

1. Introduction

It is now estimated that about nine million people develop disease caused by *Mycobacterium tuberculosis* each year.¹ Among these, the numbers of individuals infected with drug-resistant forms of the bacterium is steadily rising, and it is now estimated that the numbers of new cases of multidrug-resistant [MDR] tuberculosis may exceed 650,000.²⁻⁴ The current vaccine, BCG,⁵ provides limited protective efficacy overall and there has been a concerted effort for the last decade or more to develop new vaccine candidates, resulting in the current TB vaccine pipeline.^{6, 7} Clinical trials have begun for the more advanced vaccines, which is a major advance for the field, but with little success so far.⁸

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Ethical Approval: These studies were approved by the Institutional Animal Care and Usage Committee at Colorado State University

Conflict of interest: None

Prophylactic vaccines are of obvious priority, and for this reason the development of therapeutic vaccines, i.e. vaccines that could be given after the infection has already been acquired, has been much slower. Such “post-exposure” vaccines would be useful, but their development and design pose real challenges, both in the demonstration of efficacy, and also in terms of their safety.⁹ Their target antigens are also a major issue, as our own earliest studies demonstrated.¹⁰

Given the increasing occurrence of drug resistance of many clinical isolates, and the weak efficacy of BCG in various situations, therapeutic vaccines may be the only solution. An added concern is the potential spread of MDR strains as a deliberate act, given the recent description of isolates in Iran which appear to be completely drug-resistant.^{11, 12} Even if the exposure is accidental, this is of little comfort, and such events have already occurred among airline passengers. For these reasons, *M.tuberculosis* is listed as a Category C biodefense pathogen.

The rationale underlying the studies described here was based on the question of what antigens the bacillus might be making as it first encounters the full force of acquired immunity. There is no doubt that this profile changes, much of it due to triggering of the DosR regulon response. While the general concept in the field is that this is a prelude to establishing latency,¹³ we have questioned this concept and instead have proposed that the bacteria are undergoing physical adaptation mechanisms required to allow persistence in developing necrosis.¹⁴ One of the events at this time is an attempt by the organism to sequester/acquire metal ions, particularly ferric iron and copper.^{15, 16} In fact, areas in the developing lesions of guinea pigs that still contain large numbers of bacilli¹⁷ stain strongly with Prussian blue, indicating iron accumulation.¹⁵

Given these observations we began to focus on antigens involved in iron sequestration, as well as antigens produced by the bacterium under hypoxic conditions and a bacterial lipoprotein that is a strong TLR2 agonist, as potential therapeutic vaccines. However, to raise the bar even higher, we decided that post-exposure vaccine studies would only have relevance if tested against high virulence clinical isolates the exposed individual might encounter.

We show here that vaccines based on iron accumulation proteins, and antigens produced under stress or hypoxia, when delivered in a potent new synthetic adjuvant did not have any significant effect on the bacterial load in the lungs of guinea pigs. Despite this, vaccinated animals lived longer, and if boosted survival time was significantly increased. Analysis of the pathology of the lungs of these animals seems to support the hypothesis that the vaccines were reducing or preventing dissemination from primary lesions in the lungs, given the almost complete absence of secondary lesions in these animals. Overall, our results indicate that the concept of giving a therapeutic vaccine that will significantly reduce the bacterial load is probably unattainable, but instead such vaccines could be used to significantly prolong survival, thus hopefully providing sufficient time to allow the offending isolate to be cultured and identified, and a drug susceptibility profile determined and applied. While these studies were conducted in the context of a biodefense situation, they have obvious wider implications.

2. Methods

2.1. Guinea pigs

Female outbred Hartley guinea pigs (~500 g in weight) were purchased from the Charles River Laboratories (North Wilmington, MA, USA) and held under barrier conditions in a Biosafety Level III animal laboratory. The specific pathogen-free nature of the guinea pig

colonies was demonstrated by testing sentinel animals. All experimental protocols were approved by the Animal Care and Usage Committee of Colorado State University and comply with NIH guidelines.

2.2 Experimental infections

Strain CSU87 was obtained from our collection at Colorado State University. It is an MDR strain that grows well in both mice and guinea pigs. SAWC is a representative “typical” Beijing strain[R220 cluster] from the Western Cape region of South Africa isolated at the Division of Molecular Biology and Human Genetics at the University of Stellenbosch, and kindly provided by Dr Tommie Victor. Strain 4619 was isolated as part of a large epidemiological study in the Bay Area of San Francisco by Dr Midori Kato-Maeda, and is described elsewhere.¹⁸ All strains were grown in 7H9 broth containing 0.05% Tween-80. Thawed aliquots of frozen cultures were diluted in sterile water to the desired inoculum concentrations. A Madison chamber aerosol generation device was used to expose the animals to *M. tuberculosis*. This device was calibrated to deliver approximately 20 bacilli into the lungs. Lung bacterial loads were determined by plating serial dilutions of tissue homogenates on nutrient 7H11 agar and counting colony-forming units after 3 weeks incubation at 37°C. The infection inoculum and day 1 lung bacterial counts were determined for all the bacterial strains similarly. In survival studies, animals showing substantial weight loss with no evidence of weight rebound were euthanized. The results shown in the survival studies are based upon 6-9 guinea pigs per group; these were analyzed by Kaplan Meier statistics.

2.3. Histological analysis

The lung lobes, spleen and mediastinal lymph nodes from each guinea pig were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS). Sections from these tissues were stained using haematoxylin and eosin. The concurrent progression of lung and lymph node lesions was evaluated using a histological grading system.¹⁹

2.4 Production of antigens

DNA encoding selected *M. tuberculosis* genes, Rv1738, Rv1909, Rv2032, Rv2359, and Rv2711, Rv3130, and Rv3841 was PCR amplified from *H37Rv* genomic DNA using *Pfx* DNA polymerase (Invitrogen, Carlsbad, CA). PCR primers were designed to incorporate specific restriction enzyme sites 5' and 3' of the gene of interest and excluded in the target gene for directional cloning into the expression vector pET28a (Novagen, Madison, WI). After PCR amplification, purified PCR products were digested with restriction enzymes, ligated into pET28a plasmid using T4 DNA ligase (NEB), and then transformed into XL10G cells (Stratagene). Recombinant plasmid DNA was recovered from individual colonies grown on LB agar plates containing Kanamycin antibiotic and sequenced to confirm the correctly cloned coding sequence. The recombinant clones contain N-terminal six-histidine tag followed by a thrombin cleavage site and the *M. tuberculosis* gene of interest.

Recombinant plasmids were transformed into the *E. coli* BL21 derivative Rosetta2(DE3) (pLysS) (Novagen). Recombinant strains were cultured overnight at 37°C in 2X yeast tryptone containing appropriate antibiotics, diluted 1/50 into fresh culture medium, grown to mid-log phase (optical density at 600 nm [OD₆₀₀], 0.5 to 0.7), and induced by the addition of 1 mM IPTG. Cultures were grown for an additional 3 to 4 h, cells were harvested by centrifugation, and the bacterial pellets were stored at -20°C. Bacterial pellets were thawed and lysed by microfluidization in 20 mM Tris (pH 8.0), 150 mM NaCl, 1 mM PMSF, followed by centrifugation at 16,000 × g for 30 minutes to fractionate the soluble and insoluble material. Recombinant His-tagged protein products were isolated under native (Rv1909, Rv2359, and Rv2711) or resuspended in 8 M urea and purified under denaturing

conditions (Rv1738, Rv2032, Rv3130, and Rv3841) using Ni-nitrilotriacetic acid metal ion affinity chromatography according to the manufacturer's instructions (QIAGEN, Valencia, CA). Protein fractions were eluted with an increasing imidazole gradient and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Subsequent purifications methods were employed depending on the level of purity and residual endotoxin levels observed after affinity column purification. Methodologies employed included further ion exchange chromatography, separation based on hydrophobic interaction chemistry, or additional rounds of affinity column purification. The final purified protein fractions were combined and dialyzed against 20 mM Tris pH 8.0 buffer, 0.2 μ m filter sterilized and then concentrated using Amicon Ultra 10-kDa-molecular-mass cutoff centrifugal filters (Millipore), and quantified using the BCA protein assay (Pierce, Rockford, IL). LPS contamination was evaluated by the Limulus amoebocyte lysate assay (Cambrex Corp., East Rutherford, N.J.). A final cutoff value of less than 100 EU/mg was achieved for all of the antigens.

2.5 F57 fusion production

The DNA sequences for the three *M. tuberculosis* H37Rv strain genes related were analyzed for restriction enzyme sites and then combined to generate a 1575 base pair gene with an amino-terminal 6-histidine tag followed by the gene sequences for Rv1909, Rv2359 and Rv2711 separated 6 base pair restriction site linkers. The gene sequence was then codon optimized for expression in *E. coli* and de novo synthesized by Operon Biotechnologies in two parts into pBluescript vectors and shipped to IDRI. The two plasmids containing F57 gene fragments were then digested with NdeI and SalI, and SalI and HindIII restrictions enzymes respectively and both fragments were gel purified and ligated into NdeI-HindIII digested pET29a expression vector in a three part ligation. The resulting plasmid pET29a-F57 was sequence verified to contain the 1575 bp fragment encoding a 522 amino acid sequence with a molecular weight of 57.25 kDa. The F57 fusion protein was expressed in *E. coli* host BL-21plysS grown in 2xYT media at 37°C. Expression was induced with 0.5 mM IPTG at an OD 0.6 and growth continued for 16 hours at 23°C. Cultures were centrifuged at 6000 $\times$ g, the media supernatant discarded, and the cell pellet was resuspended in lysis buffer (20mM Tris pH8, 150mM NaCl, 5 mM Imidazole, 1% glycerol, 1mM PMSF) and stored at -20°C. Cell pellets were thawed, lysed by microfluidization, and spun at 10,000 $\times$ g for 20 minutes. The F57 fusion protein remained in the soluble fraction and was purified under native conditions. The soluble supernatant containing the F57 protein was passed through a 0.45 μ m filter and added to a 10 ml bed volume of Ni-nitrilotriacetic acid (NTA) metal ion affinity chromatography matrix according to the manufacturer's instructions (QIAGEN, Valencia, CA) and rotated for 4 hours at 4°C in a batch preparation in a 50 ml conical vial. The NTA resin centrifuged at 3000 $\times$ g for 5 minutes to pellet the NTA resin and the supernatant was removed by pipetting. The resin was then washed with 10 column volumes of wash buffer (20mM Tris pH8, 150mM NaCl, 15 mM Imidazole, 1% glycerol) followed by 10 column volumes 60% isopropanol, 20 mM Tris pH 8.0 and then re-equilibrated with 10 column volumes of wash buffer. F57 protein fractions were eluted with an increasing imidazole gradient 20-300 mM using an AKTA Explorer FPLC system and the peak F57 elution fractions (~250mM) analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Affinity-purified protein fractions were combined and dialyzed against 20 mM Tris, pH 8.0 overnight at 4°C and loaded onto a HiQ sepharose ion exchange column. The HiQ column was washed with 10 column volumes of 20 mM Tris pH 8.0, 25 mM NaCl and then eluted with a 50-500 mM NaCl gradient and the F57 protein peak elution fractions (100-200 mM NaCl) were analyzed by SDS-PAGE. F57 containing fractions were pooled and dialyzed overnight at 4°C into 20 mM Tris pH 8.0 and then concentrated to 1.0 mg/ml using Amicon Ultra 10-kDa-molecular-mass cutoff centrifugal filters (Millipore) and the concentration quantified using OD₂₈₀ nm

spectroscopic analysis and re-verified using the BCA protein assay (Pierce, Rockford, IL). Residual LPS contamination was evaluated by the Limulus amoebocyte lysate assay (Cambrex Corp., East Rutherford, N.J.) and determined at less than 25 EU/mg protein. Rv1411 was over-expressed in *M.smegmatis* to ensure correct acylation and glycosylation[needed to trigger via TLR2] as previously described.²⁰

2.5 Vaccinations

Guinea pigs were vaccinated by intramuscular injection of 10µg of each antigen, or 20µg of F57, delivered in 50µl GLA-based adjuvant suspended in 100ul saline. The adjuvant formulation consisted of 20 µg GLA plus 25µg CpG[CpG 2395; Coley Pharmaceutical Group, Inc] delivered in a stable oil-in-water emulsion[“GLA adjuvant”]. The “3-iron” antigen pool consisted of Rv1909, Rv2359, and Rv2711. The “hypoxic” pool consisted of Rv1738, Rv2032, Rv3130, and Rv3841. In several experiments we have not seen any statistically significant differences between adjuvant only controls and saline only controls, so the latter was omitted here.

3. Results

3.1. Evaluation of lung bacterial burdens after post-exposure vaccination

In a first study[Table 1] guinea pigs were infected with strain CSU87 and vaccinated with the “3-iron” pool ten days later. At day-40 of the study there was a significant drop in the bacterial load in the lungs[P<0.03]. In all subsequent studies however, including study-4 in which boosting was attempted, we were unable to significantly achieve this effect in studies using three high virulence Beijing strains.

3.2. Effect of inoculation of guinea pigs on lung pathology

We examined the pathology of the lungs in study-1 at day 40 and 90[Fig.1]. By the latter time point many of the animals given adjuvant only had died. We observed primary lesion necrosis in both sets of animals, although this appeared to be diminished in the vaccinated animals. We also noticed that the animals receiving the vaccine had very little secondary lesion development. As a result, in the absence of these, which can be large and consolidating, significant areas of the lung remained clear.

In a further study we compared the “3-iron” protein pool with a fusion protein [F57/GLA] made up of its three components[Fig.2]. In this study we infected guinea pigs with the high virulence Beijing strain 4619. On day-40 we could not see any differences between animals given the “3-iron” pool in GLA adjuvant versus animals given adjuvant only[Fig. 3]. In contrast vaccination with F57 reduced the number of primary lesions, although these were still all necrotic. In this latter group there was no evidence of lung-consolidating secondary lesion development, and these animals lived longer[P=0.048].

3.3. Effects of vaccination followed by boosting

We then addressed the question as to whether boosting might help reduce the lung bacteria load. Again, we were unable to achieve this[Study-4, Table 1] in guinea pigs vaccinated on day-10 and boosted on day-25, following infection with the high virulence Western Cape Beijing strain SWAC954. However, while lung burdens were similar, there was less necrosis in the “3-iron” vaccinated animals, and very little evidence of secondary lesion development[Fig.4]. In animals vaccinated with the hypoxia pool or with Rv1411, necrosis of primary lesions was still prominent, but again there was much less overall lung consolidation due to lack of secondary lesions. As yet, we have not been able to investigate the effects of boosting with F57.

As before, improved lung pathology resulted in prolonged survival of the vaccinated animals[Fig. 5], and in these studies we achieved statistical significance[“3-iron”, $P<0.03$; Rv1411 $P<0.02$, hypoxia pool $P<0.007$].

We elected to curtail this study at day-200. At this time there were still several survivors, and these appeared healthy and had not lost weight. We euthanized these animals and observed that while the lung tissues contained multiple areas of granulomatous inflammation there were significant reductions in total lung involvement. Moreover, many of the necrotic lesions we observed earlier had been replaced by calcification and fibrosis, indicating lesion resolution, a process evident for all three vaccine candidates tested. In addition, in the group vaccinated with the “hypox pool” there was evidence of airway epithelium regeneration. Representative lung fields are shown in Fig.6, compared to the lungs of the final survivor in the adjuvant control group which died on day-152. In that animal there was characteristic coalescing foci of granulomatous inflammation with central necrosis in multiple lesions.

These observations were consistent with body weight and lung bacterial burden obtained from these animals[Fig.7]. Whereas adjuvant control animals lost in the range of 80-140g before being euthanized, surviving animals in all three test groups appeared in general to have slightly gained weight. When we determined the lung CFU values in these animals, they were significantly lower than seen in the last adjuvant control animal, and in the cases of Rv1411 and the “hypoxic pool” there was evidence for an actual significant reduction in these values. While one must be cautious given the low statistical power here, the data does suggest that these animals had been able to substantially reduce the numbers of surviving bacteria in their lungs.

4. Discussion

The results of this study show that vaccination of guinea pigs with three therapeutic vaccine candidates given ten days after challenge infections with high virulence clinical isolates with one exception failed to significantly reduce the bacterial load in the lungs. Despite this, the animals lived longer, and this survival became statistically significant when a boosting vaccine was given two weeks later. Examination of the pathology of the lungs of these animals revealed differences between vaccinated guinea pigs and animals given the GLA adjuvant by itself. In most cases the numbers of lesions in control and test animals did not differ to any degree, but in some cases the degree of necrosis was reduced, and in most animals we noted the almost complete absence of secondary lesions. We hypothesize therefore that the vaccines are causing this, and while these animals still eventually died their survival was significantly lengthened, which in terms of human therapy would be beneficial by allowing time for more accurate diagnostic results to inform treatment decisions.

In animals given adjuvant alone, primary lesions start to become obvious 10-15 days after infection, and by 30-days of the infection most of these lesions become increasingly necrotic. At this point some of the bacilli clearly disseminate, causing the development of often very large but non-necrotic secondary granulomas.²¹⁻²³ Classically, these events are thought to be due to “hematogenous dissemination”²⁴ of the bacteria, but our more recent viewpoint is that this is equally likely to be due to carriage down lymphatics given the distribution of these lesions more towards the pleura[i.e. following the lymphatics chain].

The development of primary lesion necrosis appears to be the critical factor, in that even very early administration of the vaccines could not prevent it, nor could the vaccines slow the increasing bacterial load, and our attempts to give these vaccines to animals later than about 20-days after infection completely failed [data not shown], as did our first vaccination

studies in mice where we waited even longer.¹⁰ Obviously, for a formulation to work as a therapeutic vaccine it must be targeting antigens made by the bacterium at that time, and thus our results seem to support the idea that the bacilli are responding to initial acquired immunity by switching to proteins needed for survival as the oxygen tension drops in necrotizing lesions, and attempting to acquire ferric iron to support enzyme systems.^{15, 25} For this reason other [more immunodominant] antigens work minimally if at all, and BCG vaccination therapeutically is highly detrimental.²⁶

We previously showed²⁷ that F36, a fusion of correctly acylated Rv1411 and ESAT-6, had modest activity as a therapeutic vaccine, and in the present study we also saw improvements in pathology and survival when Rv1411 was given in GLA adjuvant. We have no way of currently telling if this was an antigen-specific effect, and in fact we suspect it is not since this protein given in GLA adjuvant would combine a potent TLR2 agonist with an adjuvant that triggers both TLR4 and TLR9. This strategy could potentially be used to enhance the vaccinogenicity of other candidates.

There are three types of “therapeutic vaccines”. The first are rapid use vaccines that could be used in emergency biodefense situations, the purpose of our studies here. A second type are vaccines that could be potentially used in conjunction with chemotherapy to try to accelerate clearance, and a third type are formulations designed to be given after chemotherapy has ceased to try to prevent relapse and reactivation disease. In terms of vaccines that could augment chemotherapy several candidates are under development, although we caution that [to our knowledge] all have been tested against laboratory strains and none so far against high virulence strains as here. The H56 fusion has been shown to slow the re-growth of H37Rv in a model of incomplete chemotherapy,²⁸ while fusion ID93 has been tested in SWR mice which were previously shown to be highly susceptible to *M.tuberculosis* infection.²⁹ In this recent study³⁰ SWR mice given RH chemotherapy lived much longer than controls, but the infection grew back and the mice died about 200-days after initial infection. The ID93/GLA-SE vaccine, when given either in conjunction with or after drug therapy, significantly increased the survival of these animals, with only about 50% mortality in these mice even 400-days after infection.³⁰ Clinical improvements were also observed in similar studies in macaques. Other candidates include RUTI,³¹ which can depress regrowth after chemotherapy has ceased, and *M.indicus pranii*.³² We ourselves have preliminary results in guinea pigs infected with CSU87 that show that day-10 vaccination given with a “ten day emergency” therapy with bedaquiline has additive effects, and this would be a reasonable emergency option if the identity of the infection was not immediately known, given the potency of this drug against MDR isolates.³³

In our early studies on this topic we were anticipating that therapeutic vaccines could be developed that could significantly reduce the bacterial load, while avoiding some obvious safety issues.⁹ Our more recent studies, in a realistic animal model infected with isolates representing reality unfortunately show this scenario is probably unlikely, since it seems that by as early as 10-15 days most of the bacilli are beginning to be out of reach of the immune response due to their increasing sequestration within primary lesion necrosis, as we have shown.^{14, 17} Our studies suggest however that post-exposure vaccination seems to have the capacity to stop bacterial dissemination, possibly by the process of lymphangitis,²⁵ preventing large and lung consolidating secondary lesions from forming. Encouragingly however, our boosting study indicated that the damaging lung pathology induced by the high virulence strains used here could be reversed to some degree in at least a percentage of our test animals, with some even showing reductions in bacterial load. This was associated with significantly improved survival times, and this effect could be of practical usefulness in that it would provide enough time to allow the identification of appropriate chemotherapy.

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Figure 1.

Lung pathology in guinea pigs after challenge with the MDR strain CSU87. Vaccinated animals were given the “3-iron” pool by intramuscular injection delivered in GLA adjuvant ten days after low dose aerosol with CSU87, and histology assessed by H&E staining at days 40 and 90. By day-90 half of the animals had already died in the adjuvant control group. Both sets of animals showed primary lesion necrosis but this was reduced in the vaccinated animals, and in addition this group showed very little secondary lesion development.

Figure 2. SDS-PAGE verification of F57. A. Graphical representation of ID57 gene construct containing Rv1909, Rv2359, and Rv2711. B. SDS-PAGE: lane 1 Molecular weight markers (kDa); lanes 2 and 3: 5 and 10 μ g of purified ID57 protein, respectively.

Adjuvant

3-iron

F57

Figure 3.

Lung pathology in guinea pigs infected with the high virulence Beijing strain 4619. In this study vaccination with the “3-iron” pool in GLA adjuvant had little effect on the lung pathology compared to adjuvant alone. In contrast vaccination with F57 reduced the number of primary lesions, although these were still all necrotic. In this group there was no evidence of lung-consolidating secondary lesion development. H&E staining.

Adjuvant

3-iron pool

Rv1411

Hypox pool

Figure 4.

Lung pathology in guinea pigs vaccinated on day-10 and boosted on day-25, following infection with the high virulence Western Cape Beijing strain SWAC954. Although lung burdens were similar, there was significantly less necrosis in the “3-iron” vaccinated animals, and very little evidence of secondary lesion development. In animals vaccinated with the hypoxia pool or with Rv1411, necrosis of primary lesions was still prominent, but there was much less overall lung consolidation due to lack of secondary lesions. H&E staining.

Figure 5.

Kaplan Meier survival analysis of guinea pigs vaccinated and boosted as above following infection with SAWC954. All three candidates all significantly prolonged survival["3-iron", $P < 0.03$; Rv1411 $P < 0.02$, hypoxia pool $P < 0.007$].

Figure 6. Lung pathology in the final adjuvant control on day-152 compared to surviving animals in the other groups, assessed on day-200. There were still substantial areas of inflammation in the surviving animals, but substantial reductions in necrosis and evidence of lesion healing and fibrosis. Low power whole organ scan, H&E staining.

Figure 7.

Animal body weight and lung CFU in animals surviving to day-200 of the study. [Left] Data shows weight at the beginning and end of the study for individual guinea pigs. [Right] Individual lung CFU values, day-200. The dotted line represents the CFU level observed in the final surviving adjuvant control animal at day-152.

Table 1

CFU evaluations after post-exposure vaccination

Study	Target	Vaccination	Day-40 CFU log±SEM]
1	CSU87MDR	Adjuvant	5.80 [0.05]
		Rv1909/Rv2711/Rv2359	5.20 [0.1] ^a
2	SF4619 W-Beijing	Adjuvant	6.66 [0.11]
		Rv1909/Rv2711/Rv2359	6.48 [0.22]
		F57 fusion	6.38 [0.19]
3	R3180 W-Beijing	Adjuvant	6.70 [0.2]
		F57 fusion	6.99 [0.22]
4 ^b	SAWC954 W-Beijing	Adjuvant	5.60 [0.2]
		Rv1909/Rv2711/Rv2359	5.66 [0.15]
		Rv1411 [acylated]	5.77 [0.06]
		Rv3841/Rv2032/Rv1738/Rv3130	6.07 [0.21]

^a: P<0.03^b: boosting study, vaccines given at day 10 and 25