Published in final edited form as:

J Med Primatol. 2011 August ; 40(4): 233–243. doi:10.1111/j.1600-0684.2011.00485.x.

Reactivation of latent tuberculosis in rhesus macaques by co-infection with Simian Immunodeficiency Virus

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Abstract

Background—Tuberculosis (TB) and AIDS together present a devastating public health challenge. Over three million deaths every year are attributed to these twin epidemics. Annually, ~ 11 million people are co-infected with HIV and *Mycobacterium tuberculosis* (*Mtb*). AIDS is thought to alter the spontaneous rate of latent TB reactivation.

Methodology—Macaques are excellent models of both TB and AIDS. Therefore, it is conceivable that they can also be used to model co-infection. Using clinical, pathological and microbiological data we addressed if latent TB infection in rhesus macaques can be reactivated by infection with Simian Immunodeficiency Virus (SIV).

Results—A low-dose aerosol infection of rhesus macaques with *Mtb* caused latent, asymptomatic TB infection. Infection of macaques exhibiting latent TB with a rhesus-specific strain of SIV significantly reactivated TB.

Conclusions—Rhesus macaques are excellent model of TB/AIDS co-infection and can be used to study the phenomena of TB latency and reactivation.

Keywords

M. tuberculosis; TB/AIDS co-infection; nonhuman primate; macaque; latency; reactivation

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AUTHOR CONTRIBUTIONS

Funding - DK, AAL, RSV; Research design - DK, SM; Research - SM, NAG, NKD, MA, CJR, XA, RSV; Data analysis: DK, SM, CJR, AAL; Veterinary medicine: LAD, KRL, JLB; Veterinary pathology: PJD, AAL, Writing - DK and SM.

The authors declare no conflict of interest in connection with this manuscript. All procedures related to *Mtb* were approved by the Tulane Institutional Biosafety Committee. All procedures involving animals were reviewed and approved by the TNPRC Institutional Animal Care and Use Committee.

INTRODUCTION

TB and AIDS are responsible for the death of over three million people every year (1, 2). One third of the world population is latently infected with *Mtb*. About 1.7 million people die of TB each year (1). The situation is equally grim on the AIDS front. An estimated 5 million people are infected with HIV every year (3). Currently there are no promising AIDS vaccines. Recently, the candidate AIDS vaccine that had generated the most hope, failed in clinical trials (4). An estimated 11 million people are co-infected yearly with HIV and *Mtb* (5). The emergence of drug resistant strains has compounded this situation (6-8). In hotbed areas, an estimated 40% of all TB patients are MDR and 10% of all patients are XDR, a majority of which are also HIV⁺ (9). It is believed that the deficiency of activated CD4⁺ T cells - which play a key role in the containment of the pulmonary *Mtb* infection - is responsible for the increased rates of TB in the post-AIDS world (10). However, the molecular and immunological mechanisms of how both pathogens impact each other are not well understood.

Animal models of infectious disease are necessary to understand mechanisms of pathogenesis and to pre-clinically predict the value of candidate vaccines and drugs. Animal models should mimic human disease as closely as possible. Mice, guinea pigs, rabbits and NHPs have all contributed to our understanding of TB (11-16). Mice have been instrumental in enhancing our understanding of TB, e.g. the role of *Mtb* virulence factors (11), and the role of various components of the immune system in protection from *Mtb* infection (17-19). Although easy to use and inexpensive, mice do not sufficiently model critical aspects of human TB. All infected mice eventually succumb to infection, as opposed to a small fraction of immunocompetent humans. Mice also fail to develop necrotic lesions, the precursors of the cavities that characterize late-stage human TB (20). There are advantages as well as limitations to both guinea pig (12) and rabbit (13) models. Immunologic reagents for both these models are scarce. NHPs, such as the rhesus (*Macaca mulatta*) or the cynomolgus (*Macaca fascicularis*) macaques have been sparingly used for TB research due to issues like handling, space, availability and expense. However, experimentally infected macaques most accurately reflect human TB in clinical and pathological contexts. They develop acute fatal cavitary or chronic disease based upon the challenge dose (14-16, 21-23). Immunological and molecular reagents are readily available for monkeys due to cross reactivity with humans and their extensive use for studying AIDS. Macaques allow the study of aspects of human TB that are difficult to model in other animals, such as pathology, latency and various outcomes.

Upon infection with a very low dose of highly pathogenic *Mtb*, cynomolgus macaques either progress to advanced disease, show chronic infection or exhibit latent infection (no signs of clinical disease) (15, 21). In this model, depletion of TNF as well as co-infection with SIV can cause reactivation of TB (24, 25). Recently, we have reported that rhesus macaques infected by exposing them to high-doses of *Mtb* aerosols develop a rapidly fatal pulmonary TB (22, 23). This model has been used to study both the bacterial (22) as well as host mediators (23) involved in TB, using high throughput approaches. The key difference between this study and the previous ones is that we infected NHPs with *Mtb* via the aerosol mode. Natural infection with *Mtb* involves exposure to infectious aerosols. Hence, aerosol mediated infection of NHPs represents the most natural and accurate model to study TB. This method also subjects *Mtb* to environmental and nutritional stresses comparable to those experienced during the infection process. It is conceivable that such stress primes *Mtb* for successful infection of primate lungs.

Rhesus macaques have been instrumental in our understanding of the pathogenesis of AIDS (26). HIV and SIV are closely related lentiviruses that cause AIDS in humans and

macaques, respectively, with remarkably similar disease progression. Using rhesus macaque adapted strains of SIV it is possible to model a rapid loss of CD4⁺ cells in the periphery as well as lymphoid organs (27), leading to immunodeficiency in a majority of animals.

In spite of the extensive synergy between TB and AIDS that is apparent from clinical and epidemiological data, co-infection of the two pathogens has never been studied in sufficient detail. Only one recent study (25) attempted to study TB/AIDS co-infection in a macaque model. However, the host species used in this experiment was the cynomolgus macaque, which is not a natural host for SIV_{mac} viruses. We therefore designed experiments aimed at studying TB/AIDS co-infection in an aerosol infection based rhesus macaque model.

MATERIALS AND METHODS

Experimental Animals

Sixteen Indian origin adult rhesus macaques (*Macaca mulatta*) were used for these studies. The animals were obtained from the TNPRC breeding colony. Before beginning the study, the animals were quarantined for 90 days and tested by both tuberculin skin-test (TST) and an NHP Interferon-gamma release assay (PRIMAGAM) (22), to ensure they were free of prior *Mtb* infection. SIV-infected animals were housed under BSL-2 conditions while *Mtb*-infected animals were housed under BSL-3 conditions. Blood draws, bronchoalveolar lavage (BAL), bronchial lymph node (BrLN) biopsies and other procedures were performed as previously described (16, 22).

Mtb and SIV Infection

The experimental design of these studies is described in Figure 1. Twelve animals were presented with ~ 500 CFU *Mtb* CDC1551 via a head-only aerosol method as previously described (22). *Mtb* infection was confirmed in all animals by conversion to positive TST and PRIMAGAM. Animals were classified as latent if positive for TST or PRIMAGAM test in the absence of signs of clinical TB. For SIV infection, concentrated SIV_{mac239} stock was diluted in RPMI medium, and 300 TCID₅₀ units of virus injected intravenously. Six animals with latent TB and four *Mtb*-negative animals were infected with SIV, with the four *Mtb*-negative animals designated as the SIV-only control group.

Clinical measurements of latent or reactivation TB

Criteria for assessing reactivation included a combination of the following: increased body temperature, prolonged weight or appetite loss, elevated serum C-reactive protein, lung granulomatous immunopathology as observed by thoracic X-rays and by gross and histopathology post-necropsy and the ability to culture *Mtb* from broncho-alveolar lavages. These clinical measures of TB in rhesus macaques have been described in detail previously (22).

Microbiological measurements of *Mtb* and SIV from rhesus macaques

Mtb was recovered from macaque blood and BAL samples as well as from homogenized (Biospec Homogenizer, Fisher Scientific, catalog number 11-504-206) tissues (lung, BrLNs, liver, spleen and kidney at necropsy). Tissues were digested using the BBL MycoPrep Mycobacterial System Digestion/Decontamination Kit, (BD Diagnostics, catalog number B4340862), as previously described (22).

SIV-load was determined in plasma by quantitative RT-PCR. Plasma samples were spiked with armored RNA (Asurgen) and centrifuged for 1 hour at 25,000g. Viral RNA was extracted using Proteinase K (2.5 µg/µl; Life Tech), the High Pure Viral RNA kit (Roche Indianapolis, IN) and the RNA Clean and Concentrator kit (ZYMO Research). Viral RNA

was reverse-transcribed using MultiScribe™ Reverse Transcriptase (Life Tech). The resulting cDNA was added to TaqMan master mix (Life Tech) along with primers and probe targeting the gag region of SIVmac239 and subjected to 40 cycles of qPCR analyses. Fluorescence signals were detected with an Applied Biosystems 7900HT Sequence Detector. Data were captured and analyzed with Sequence Detector Software (Life Tech). Viral copy numbers were calculated by plotting CT values obtained from samples against a standard curve generated with *in vitro*-transcribed RNA representing known viral copy numbers (and controlled by addition of known copies of aRNA).

Pathology of TB and TB/AIDS co-infection in rhesus macaques

Methods pertaining to gross and histopathology analysis in the tissues of infected macaques have been described in detail previously (22). Both *Mtb* and SIV were detected in the lungs of singly and co-infected animals by fluorescently labeled antibodies as described (23, 28).

RESULTS

As shown in Figure 1A, we designed experiments to understand if SIV co-infection could reactivate latent TB infection in rhesus macaques. All animals were exposed to infectious aerosols of *Mtb* using a head-only exposure system, as previously described (23). The input dose of *Mtb* was adjusted so that approximately 500 cfu of the low-virulence strain CDC1551 would be presented to and inhaled by the animals. All the animals were examined weekly by a board certified veterinary clinician (Fig 1A). Body temperature and weight, as well as blood chemistry and CBC's were also obtained weekly during the initial phase of *Mtb* infection, and bimonthly thereafter. Periodically, BAL samples were also obtained from these animals (Fig. 1A). The experiment was designed to allow the identification of animals with latent or asymptomatic TB infection, and those which exhibited signs of reactivated TB following co-infection with SIV. Six animals were only exposed to *Mtb* aerosols (group I). Of these, one animal (EL04) exhibited signs of active TB from a very early stage post *Mtb*-infection and was excluded from further analyses. Therefore results are presented for only five NHPs in group I. Six animals were infected intravenously with SIV_{mac239} at the 9 week post-*Mtb* infection time-point and designated group II. The five group I animals received a placebo dose of PBS, intravenously. We also had access to a third group (group III) of NHPs that were simultaneously infected with a similar dose of SIV_{mac239} only.

We used tuberculin skin-test and PRIMAGAM to measure the extent of infection. All twelve animals in group I and II exhibited a positive TST (Table 1) and a positive PRIMAGAM (not shown) between 3-7 weeks post-infection. This conversion confirms that all animals were exposed to *Mtb* aerosols.

Clinical correlates of latent *Mtb* infection and reactivation

Prior to infection, all animals exhibited normal body temperatures, in the range of 98.5-100°F. We measured the temperature of group I and II animals during the course of *Mtb* infection, and then following SIV (or placebo) co-infection. These eleven animals exhibited no detectable change in temperature over the first nine weeks post-infection (Fig 2A). Animals in group II, but not group I, exhibited increased body temperatures following SIV co-infection at week 9 (Fig 2A). The differences between group I and II were statistically significant at several time-points.

Similarly, none of the eleven animals exhibited any appreciable weight-loss during the first nine weeks post *Mtb*-infection. However, most animals of group II exhibited weight-loss post SIV-infection (Fig 2B). These differences approached statistical significance with a clear trend of weight-loss in group II NHPs. All animals in this group with the exception of

one exhibited notable weight-loss (15-30% of their body weight prior to *Mtb*-infection), while none of the *Mtb*-infected animals exhibited significant weight loss (< 10% of their body weight prior to *Mtb*-infection) (Fig 2B).

We have earlier shown that the serum levels of the systemic inflammatory marker C-reactive protein (CRP) coincide with the advent of active TB. We therefore measured the levels of CRP in the two groups of animals. The serum CRP levels remained close to baseline values prior to SIV infection for most animals. However, the serum CRP levels of all group II animals exhibited a significant increase within 3-7 weeks of SIV-infection (Fig 2C). Serum CRP levels of all group I animals remained significantly lower post-placebo inoculation (Fig 2C).

We also analyzed the extent of lung involvement using chest X rays (CXRs) at different time points during infection. The CXRs were scored by veterinary clinicians on a subjective scale of 0-3, with a score of 0 denoting normal lung, and a score of 3 denoting severe tuberculous pneumonia. During weeks 7-9 post-*Mtb* infection (prior to co-infection with SIV), all 11 NHPs exhibited CXR scores of 0 or 1, consistent with the diagnosis of latent or asymptomatic TB. However, at week 12 post-*Mtb* infection (three weeks post-SIV infection), the group II animals exhibited higher CXR scores relative to those in group I (Table 2). These differences were however, not statistically significant.

Infection with a low-dose of a low-virulence *Mtb* strain caused asymptomatic TB infection in rhesus macaques (Figs 2A-C). Infection was clearly reactivated by SIV co-infection (Figs 2A-C). Reactivation of latent TB significantly reduced the survival of co-infected animals (Fig. 2D). None of the five animals in group I that progressed to asymptomatic infection by week 9 died due to TB related complications during the study. However, of the six animals from group II which were co-infected with SIV, four succumbed to TB related complications. These survival differences were significant (Fig 2D) pointing to the fact that SIV-infection related immunodeficiency reactivates latent TB infection in NHPs.

Microbiological correlates of latent *Mtb* infection and reactivation

We measured the level of *Mtb* replication in latent as well as reactivation disease in blood and BAL samples. Post-*Mtb* infection, but prior to SIV or placebo co-infection, no *Mtb* CFU's could be detected in the blood of any of the 11 NHPs in group I or II (Fig. 3A). Similarly, BAL samples of all NHPs were negative for *Mtb* during the post-*Mtb* infection phase (Fig. 3B). However, post-SIV inoculation, the animals in group II experienced a rapid increase in *Mtb* replication in lungs, as measured by CFU levels in the BAL (Fig. 3B). Surprisingly, some bacilli could also be detected in the blood of SIV co-infected NHPs (Fig. 3A). This was surprising since the CDC1551 strain is not known to disseminate. Even during high-dose aerosol infection of NHPs with this strain, we have been unable to detect *Mtb* in blood (Mehra et al, unpublished observations).

We also measured the bacterial load in BrLNs at different times during infection (Fig. 3C). At necropsy, several randomly chosen regions from both lungs were also used to determine *Mtb* load (Fig. 3D). *Mtb* could be detected in BrLNs of all animals and the loads were statistically indistinguishable between the two groups (Fig. 3C). However, a statistically significant higher number of *Mtb* CFUs could be detected in the lungs of group II animals (Fig. 3D). While the total *Mtb* burden per gram of tissue was higher in BrLNs than in lungs, the replication of *Mtb* was significantly increased in lungs by SIV co-infection. Similarly, a higher number of *Mtb* CFUs could be cultured from the spleen (Fig. 3E), liver (Fig. 3F) and kidneys (not shown) of the group II (SIV co-infected animals) relative to group I (singly infected animals). Taken together, the CFU results indicate that asymptomatic TB was reactivated upon co-infection with SIV.

We also measured the SIV loads present in the plasma of animals in group II in the weeks following inoculation with SIV (Fig 3G). These results were compared to animals in group III, which only received an equivalent dose of SIV_{mac239}. The levels of SIV in the plasma from the two groups of animals were not significantly different, indicating that the course of SIV replication was not influenced by either the presence of a latent TB infection or its reactivation.

Pathology of latent *Mtb* infection and reactivation

At necropsy, various tissues such as lungs, associated lymphatics, spleen, liver and kidneys were grossly analyzed for the presence of tuberculous lesions in both groups of animals. A few discrete lesions could be observed in the lungs and lymph nodes of group I animals. However, no lesions were present in the extra-thoracic tissues of the NHPs from group I. In contrast, significantly higher lesion counts were visible in the lungs and lymph nodes of the group II animals. These animals also exhibited numerous discrete lesions in spleen, liver and kidney. Representative gross and histopathology lung images for an animal from group I (Fig. 4A and 4B) and group II (Fig. 4C and 4D) are shown. Representative images from the spleen (Fig 4E) and liver of a co-infected (group II) NHP (Fig 4F) are shown. These images clearly show tuberculous lesions in these extra-thoracic tissues, relative to the corresponding, completely normal tissues from the NHPs infected with low-dose *Mtb* (not shown).

Using confocal microscopy, we studied the presence of SIV in the lung tissues of co-infected animals. We were able to detect the presence of both *Mtb* and SIV using specific antibodies. Interestingly, SIV along with *Mtb* appeared to infected macrophages in the lesions obtained from lung as well as lymph nodes. Both in lung (Fig. 5A) and BrLN (Fig. 5B) we were able to detect the co-localization of both *Mtb* and SIV in the same cells. Additionally, numerous virus-positive cells were located in the vicinity of *Mtb*-positive cells. This result clearly shows that in-spite of a systemic (intravenous) route of inoculation SIV is able to pathogenize lungs and co-infect the same cells *Mtb*.

DISCUSSION

It is widely believed that reactivation of latent TB infection in AIDS patients is the single largest reason behind the resurgence of TB as a major cause of human mortality. However, there are only a limited number of studies that have attempted to address this question in a research setting. In part the problem stems from the lack of effective experimental models of co-infection. Mice, the predominant choice of animal system to study *Mtb* virulence factors as well as the role of host immune mediators in the pathogenesis of the disease, can't be used to model AIDS. NHPs, particularly the Indian rhesus macaque, are an excellent model for studying the pathogenesis of human AIDS (26, 27). Over the last decade, there has been renewed interest in using NHPs to model TB as well (15, 16, 21-25). Both cynomolgus and rhesus macaques have been used to model latent as well as acute TB infections (15, 16). The cynomolgus macaque model has allowed a better understanding of the role played by TNF (24) and regulatory T cells (29) during TB. The rhesus macaque model has allowed the discovery of *Mtb* genes essential for virulence in primates (22), as well as study host gene-expression (23). Therefore, NHPs appear to be an excellent system for modeling TB/AIDS co-infections. We believe that the model can be used to study the host factors that could lead to reactivation of latent TB infection.

In our studies, we used rhesus macaques as the primate host for latent TB infection. We have already shown that this species can be infected with a high dose of *Mtb* aerosols (22, 23). We used a highly immunogenic, low-virulence strain of *Mtb* (CDC1551). We have previously noted that that CDC1551 strain exhibits significantly low virulence as compared

to the *Mtb* Erdman strain (Mehra et al, unpublished observations). In order to generate latent infection with the Erdman strain, as few as 25-50 bacilli may be required. This number of *Mtb* CFU is difficult to successfully obtain repeatedly when using the aerosol technique. The use of CDC1551 allowed us to perform low-dose aerosol infections with as many as ~ 500 CFU. After a period of time where a large majority of NHPs developed evidence of asymptomatic, latent TB infection, as characterized by positive TST and PRIMAGAM tests, we co-infected one group with SIV_{mac239}, a pathogenic strain of SIV. This is a rhesus macaque adapted strain that is known to generate AIDS related immunodeficiency in this species. The dose used (300TCID₅₀) was in itself nonlethal. This dose was also significantly (about four logs) lower than the dose of SIV (10⁶ TCID₅₀) used by Dietrich et al (25) to generate reactivation of latent TB in cynomolgus macaques and thus represents a more physiologically relevant dose.

Most of the NHPs that were co-infected with SIV significantly reactivated TB infection, as assessed by clinical, pathological and microbiological measures. These included evidence of dissemination of *Mtb* to extra-thoracic tissues such as spleen, liver and kidney. Clinically, these animals exhibited a higher level of systemic inflammation, as judged by significantly higher serum C-reactive protein values. A statistically significant reduced survival of the co-infected animals, relative to *Mtb*-infected animals also points to the reactivation of asymptomatic TB infection. In stark contrast, none of the *Mtb*-only infected animals showed any signs of active TB during the relative time period, as judged by clinical, microbiological and pathological correlates. In the only animal from this initial group of six NHPs which failed to generate latent TB infection, the signs of active TB were apparent from as early as week 3 post-*Mtb* infection.

Our study suggests that even at a physiologically relevant low-dose, SIV infection rapidly and definitively reactivates latent infection with a low-virulence strain of *Mtb* in rhesus macaques. These results have far reaching consequences for understanding the immunology of co-infection. The use of a low-dose of SIV to rapidly reactivate latent *Mtb* infection in rhesus points to the fact that this species is an excellent model for studying the immunological markers of TB/AIDS co-infection. We have already described the system-wide expression of host genes in early and relatively mature TB granuloma lesions (23). In future we would like to study if gene-expression signatures in the granulomatous lesions induced by SIV-mediated reactivation are similar to or different from those induced by active TB. We are also using this model to study the recruitment of and the activation status of different immune cells, such as macrophages, lymphocytes and neutrophils, to the site of pulmonary infection.

Using multi-label confocal microscopy, we have shown that SIV is able to infect lungs and BrLNs of *Mtb*-infected NHPs, despite its systemic route of inoculation. Further we show co-localization of both pathogens in the lungs as well as BrLNs of the same animals. This is perhaps the first direct illustration of co-infection by *Mtb* and SIV (or possibly HIV), at the cellular and molecular level. Using this model and the multi-labeling technique, we would in future like to address the hypothesis that SIV infection interferes with the clearance of *Mtb* infection by modulating anti-tuberculocidal functions of the infected and bystander macrophages.

Acknowledgments

This work was funded by the NIH grants RR026006 and AI091457 (both to DK) and supported by NIH grants AI089323, HL10679 (both to DK), AI084793 (to RSV), RR000164 and RR020159; and awards by Louisiana Vaccine Center (to DK), Tulane Research Enhancement Fund (to DK) and Tulane Center for Infectious Diseases (to SM). We acknowledge Dr Preston Marx for providing a stock of SIV_{mac239}; Stephanie Killeen and Sathesh Sivasubramani for *Mtb* infections and Mary Barnes for PRIMAGAM assay.

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Figure 1. The experimental design for *Mtb*/SIV co-infection in NHPs (A)

All animals were infected with ~ 500 CFU *Mtb* CDC1551. PE 1, PE 2 and PE 3 correspond to the different collection of clinical activities performed on the two groups of animals at different time-points, including prior to *Mtb* infection. 300 TCID₅₀ SIV_{mac239} in PBS buffer was introduced i.v. into six NHPs belonging to group II. The group I animals received placebo (PBS).

Figure 2. Clinical correlates of latent TB and reactivation

We studied change in body temperature, expressed as change in °F (A), change in percent body-weight (B), and serum CRP concentrations, expressed as mg/ml (C). Closed circles represent group I, while closed squares represent group II.

Comparison of the survival percentages for *Mtb*-infected and *Mtb*/SIV co-infected NHPs (D). Closed circles represent group I, while closed squares represent group II.

Figure 3. Microbiological correlates of latent TB and reactivation

Mtb CFUs recovered from blood (A) and BAL (B) during different time periods of infection, both expressed as CFU/80 mL. *Mtb* CFUs at necropsy in BrLN (C), lung (D), spleen (E), liver (F) and kidney (G), expressed as CFU/gram of tissue. Plasma SIV viral copy number as assessed by quantitative RT-PCR (H), prior to and after SIV co-infection. Data is shown for NHPs in group II (closed squares), as well as group III (open squares) for control plasma (prior to SIV infection) and at weeks 10, 12 and 13 post *Mtb*-infection (i.e. weeks 1, 3 and 4 post-SIV infection) for both groups.

Figure 4. Pathology of latent and reactivation TB

Representative gross pathology images from the lungs of an NHP from group I (A) and II (B). Representative H&E stained images from the lungs of an NHP from group I (C) and II (D).

Figure 5. Co-localization of *Mtb* and SIV in the tissues of co-infected animals

Multi-label confocal microscopy was used to identify if *Mtb* and SIV co-localize in the lungs (A) or BrLNs (B) of co-infected animals. A representative image from each tissue is being shown. Specific signal was detected under differential interference contrast (DIC). SIV was detected by using a p28-specific antibody raised in mouse (Microbix Biosystems #AS-59-06) as previously described (28). *Mtb* was detected by using a polyclonal anti-*Mtb* antibody raised in rabbit (Abcam #ab905), as previously described (23).

TST results

Table 1

TST results are tabulated for all 12 animals in groups I and II prior to *Mtb* infection, and at 4, 8 and 12 weeks post *Mtb*-infection. Tests were assessed at 24, 48 and 72 hr post-administration. Negative results for each time point are represented by an N while positive results for each time point are represented by a P.

Group I	Animal	TST result (Pre-infection)	TST result (Week 4)	TST result (Week 9)	TST result (Week 12)
	NHP 1	NNN	PPN	NNN	NNN
	NHP 2	NNN	PPP	PPP	NNN
	NHP 3	NNN	N-N	NPP	PPP
	NHP 4	NNN	NPN	NPP	PPP
	NHP 5	NNN	N-N	PPP	PPP
Group II	NHP 1	NNN	NNN	PPP	NNN
	NHP 2	NNN	PPP	PPP	PPP
	NHP 3	NNN	NPN	PPP	PPP
	NHP 4	NNN	PPN	PPP	PPP
	NHP 5	NNN	PPP	PPP	PNN
	NHP 6	NNN	NPP	PPP	NNN

Chest X-rays

Table 2

Arbitrary scores from CXRs obtained from all group I and II animals are shown for the following time-points: prior to, 4, 8 and 12 weeks post-*Mtb* infection. The following subjective scoring system was used: 0 (no pneumonia); 1 (mild pneumonia); 2 (moderate pneumonia); 3 (severe pneumonia).

Group I	Animal	CXR Score (Pre-infection)	CXR Score (Week 4)	CXR Score (Week 7-9)	CXR Score (Week 12)
	NHP 1	0	1	1	3
	NHP 2	0	0	ND	1
	NHP 3	0	1	1	1
	NHP 4	0	1	1	1
	NHP 5	0	0	0	0
	NHP 6	0	0	0	0
Group II	Animal	CXR Score (Pre-infection)	CXR Score (Week 4)	CXR Score (Week 7-9)	CXR Score (Week 12)
	NHP 1	0	1	1	2
	NHP 2	0	0	1	2
	NHP 3	0	1	1	3
	NHP 4	0	0	0	1
	NHP 5	0	0	0	3
	NHP 6	0	0	0	3