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Once-weekly oral dose of isoniazid and rifapentine for 12 weeks (3HP) is recommended by CDC for treatment of latent tuberculosis infection (LTBI). The aim of this study is to assess 3HP-mediated clearance of Mtb bacteria in macaques with asymptomatic LTBI. Twelve Indian rhesus macaques were infected with low dose (~10 CFU) of Mtb CDC1551 via aerosol. Six animals were treated with 3HP and six were left untreated. The animals were imaged via positron emissions tomography – computed tomography (PET/CT) at frequent intervals. Upon treatment completion, all animals except one were coinfected with simian immunodeficiency virus to assess reactivation of LTBI to active TB disease. Four of six treated macaques showed no evidence of persistent bacilli or extrapulmonary spread until study end-point. PET/CT demonstrated the presence of significantly more granulomas in untreated animals relative to the treated group. The untreated animals harbored persistent bacilli and demonstrated TB reactivation following SIV coinfection while none of the treated animals reactivated to active TB disease (ATB). 3HP treatment effectively reduced persistent infection with Mtb and prevented reactivation of TB disease in latently infected macaques.

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Isoniazid and Rifapentine Treatment effectively reduces persistent *M. tuberculosis* infection in macaque lungs

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Abbreviations: ATB, active TB; BAL, bronchoalveolar lavage; CRP, C-reactive protein;
PET/CT: positron emission tomography/computed tomography; LTBI, latent tuberculosis
infection; Mtb, *Mycobacterium tuberculosis*; NHPs, nonhuman primates; TB, tuberculosis; TST,
tuberculin skin test.
Abstract

Once-weekly oral dose of isoniazid and rifapentine for 12 weeks (3HP) is recommended by CDC for treatment of latent tuberculosis infection (LTBI). The aim of this study is to assess 3HP-mediated clearance of *Mtb* bacteria in macaques with asymptomatic LTBI. Twelve Indian rhesus macaques were infected with low dose (~10 CFU) of *Mtb* CDC1551 via aerosol. Six animals were treated with 3HP and six were left untreated. The animals were imaged via positron emissions tomography – computed tomography (PET/CT) at frequent intervals. Upon treatment completion, all animals except one were coinfected with simian immunodeficiency virus to assess reactivation of LTBI to active TB disease. Four of six treated macaques showed no evidence of persistent bacilli or extrapulmonary spread until study end-point. PET/CT demonstrated the presence of significantly more granulomas in untreated animals relative to the treated group. The untreated animals harbored persistent bacilli and demonstrated TB reactivation following SIV coinfection while none of the treated animals reactivated to active TB disease (ATB). 3HP treatment effectively reduced persistent infection with *Mtb* and prevented reactivation of TB disease in latently infected macaques.
Introduction

Most people infected with *Mycobacterium tuberculosis* (*Mtb*) do not progress to active tuberculosis (ATB) but instead contain the bacteria and develop asymptomatic latent TB infection (LTBI) [1]. However, these individuals remain at risk for developing ATB disease, for example when co-infected with Human Immunodeficiency Virus [2]. The commercial tests available to detect LTBI including the Tuberculin Skin Test [2], and ELISA-based Interferon Gamma Release Assays (IGRAs) [3], fail to determine whether an individual has cleared infection or harbors persistent bacilli. The CDC recommends the use of once-weekly regimen of isoniazid and rifapentine for 3 months (3HP) for treatment of LTBI in humans [4]. 3HP is effective in reducing the risk of developing active TB [5], suggesting that it mediates clearance of *Mtb* in LTBI. However, the sterilizing efficacy of the regimen on *Mtb* has not been demonstrated. Thus, a better understanding of treatment-mediated clearance of *Mtb* infection is needed in order to improve monitoring and evaluation of treatment regimens for LTBI.

The nonhuman primate (NHP) model is attractive for studying human *Mtb* infection and for performing preclinical studies on treatment regimens as it recapitulates key aspects of human *Mtb* infection states and TB disease [6]. A majority of rhesus macaques infected with low dose *Mtb* CDC1551 via aerosolization develop asymptomatic LTBI [7, 8]. Moreover, co-infecting latently *Mtb*-infected macaques with simian immunodeficiency virus (SIV) results in reproducible reactivation [9]. Thus, the NHP model allows us to gain longitudinal and mechanistic insights into the efficacy of treatment regimens for *Mtb*, including in lung compartments, which is difficult to investigate in humans. Between 2014-2017, we conducted studies in the rhesus macaque model of LTBI and SIV-induced reactivation of TB to evaluate the efficacy of the 3HP regimen [10]. We discovered irregularities in the timing and frequency of treatment in a subset of the animals reported in that study which led us to subsequently retract the published work [10]. In this study, we repeated the study to investigate the persistence of *Mtb* in the lungs of asymptomatic rhesus macaques with long-term *Mtb*-infection and to evaluate the efficacy of 3HP in eradicating persistent *Mtb* in a macaque model of 3HP treatment. To assess the effectiveness of 3HP in clearing *Mtb* infection, we co-infected both treated and untreated animals with SIV. Our results clearly suggest that the 3HP treatment is highly efficacious, leading to highly significant reduction in
clinical signs of TB, bacterial burden and granuloma numbers and volume of inflammation and disease.

Results

Clinical correlates of LTBI, 3HP treatment, and TB reactivation in rhesus macaques

Twelve animals were exposed to a low dose of Mtb CDC1551 (Figure 1A). Infection was confirmed by a positive TST [2] at weeks 3 and 5 post Mtb infection. All animals in the study developed LTBI infection, characterized by the absence of culturable bacilli in bronchoalveolar lavage; BAL, serum C-reactive protein (CRP) ≤ 10 µg/mL (Figure 1B) and no significant changes in percentage body temperature (Figure 1C) and body weight (Figure 1D) for up to 12 weeks post Mtb infection. One group (n=6) remained untreated, whereas the second group (n=6) was treated with the once weekly 3HP regimen for 12 weeks. One month after treatment completion, (i.e., 7 months after Mtb infection), coinfection with SIV led to TB reactivation in the majority of untreated animals, as demonstrated by increased CRP levels (Figure 1B). One of the animals in this group (31438) progressed to active TB by week 18 (evident from increased CRP levels in this animal, Figure 1B, and significant weight loss, Figure 1D). Therefore this animal was not co-infected with SIV, and was instead euthanized 32 weeks post-TB infection (its data was included in the data analysis). Due to the clinical signs and symptoms of TB reactivation: CRP levels > 10 µg/mL, >20% weight loss, loss of appetite and increased lesions as seen via PET/CT, the control animals were humanely euthanized (Figure 1E). While 3 animals demonstrated more weight loss compared to the others during the treatment period, the weight loss was not significantly greater nor consistent for more than 2 weeks in the same animal. There was no significant difference between CRP values of untreated versus 3HP treated animals at week 3 (P = 0.91), week 9 (P = 0.61) or week 23 (0.08) post Mtb infection. However, there were significant differences in the CRP levels post-SIV co-infection at necropsy between the two groups (P = 0.01). Importantly, none of the 3HP-treated animals exhibited elevated CRP levels (Figure 1B), pyrexia (Figure 1C) or wasting (Figure 1D) after SIV co-infection and did not need to be euthanized due to disease progression (Figure 1E). These animals were subsequently euthanized for necropsy and tissue collection at week 34 post-TB infection. No significant differences were observed in blood biochemistry (Figure 1F) between the two groups following 3HP treatment completion, confirming absence of drug-induced cytotoxicity. These results indicate that a significant number of macaques in this
study were infected with *Mtb* for >28 weeks and remained asymptomatic until significant immune
perturbation occurs *via* SIV co-infection. However, it is possible that a percentage of macaques
could have reactivated had they been left untreated.

**PET/CT imaging analysis of TB reactivation**

Co-infection with SIV led to TB reactivation in untreated animals, as demonstrated by the presence
of numerous granulomatous lesions by CT scans (Figure 2A). While most lesions were present in
the solitary spontaneous reactivator animal in this group which reactivated prior to SIV co-
infection (marked with black arrow), all five untreated, SIV co-infected animals (except 33997)
had clear evidence of granulomatous lesions. Animal 36462 had comparatively less evidence of
progression. Furthermore, the 3HP-treated animals did not demonstrate the presence of increased
number of lesions after SIV co-infection (Figure 2B) (marked with black arrow). The lung lesions
in all macaques remained stable i.e. no or minimal progression in size and architecture at week 8-
10 post infection confirming LTBI (Figure 2A-B, top row) (marked with black arrow). Five out of
six macaques in the control group showed gradual progression in TB pathology with multiple new
lung lesion and an increase in size of previously shown nodular lung lesions post-SIV co-infection
(Figure 2A, bottom row) (marked with black arrow).

TB pathogenesis and efficacy of 3HP prophylaxis regimen were examined using positron emission
tomography (PET-CT) scans [11] (Figure 3). All the macaques (12/12) in the study had focal
nodular lung opacities, while (9/12) displayed mild to moderate lymph node enlargement by 5 to
6 weeks post aerosol *Mtb* infection. The 18F-fluorodeoxyglucose (FDG) scans were performed 3
weeks post completion of 3HP regimen, i.e., week 26, in all animals (Figure 3A-B). These scans
clearly revealed both the presence of persistent foci of increased FDG uptake in the controls
(Figure 3A) and the effectiveness of the 3HP regimen (Figure 3B) at the completion of the
treatment (top row). After SIV infection, scans in the treated group reported few to no new lung
lesions, while the previously shown lung lesions were resolved, i.e., no increase in lesion volume
and no increase in FDG uptake (Figure 3B) was observed in the majority of the animals in this
cohort. In contrast, five out of six control (untreated) animals showed an increase in size of lung
lesions and increased FDG SUV (Figure 3A), signifying reactivation and further progression of
lung TB pathology. All six untreated controls showed involvement of multiple lung lobes, with
some examples of consolidation, lobar collapse, cavitary lesions and massive mediastinal lymph node enlargement, post-SIV challenge. The number ($P = 0.0181$) (Figure 3C) and volume of lung lesions ($P = 0.0335$) (Figure 3D), lung lesion activity ($P = 0.0002$) (Figure 3E), SUVmax ($P = 0.0036$) (Figure 3F) and total lung activity ($P = 0.0335$) (Figure 3G) of control animals were each significantly higher compared to 3HP treatment group post treatment completion. Our results therefore clearly suggest effective resolution of lung TB lesions post prophylactic 3HP regimen.

**3HP treatment-mediated clearance of persistent Mtb infection in macaques**

To assess *Mtb* bacterial burdens in pulmonary and extrapulmonary compartments of 3HP-treated and untreated animals following SIV co-infection, lungs and other organs were assayed for *Mtb* by culture at necropsy (Figure 4). The lung bacterial CFU loads in the untreated group (mean 3.56 log) were significantly higher than in the 3HP-treated group (mean 1.0 log; $P = 0.0085$) (Figure 4A). All six of the untreated animals harbored bacilli in their lungs while four of the six 3HP treated animals were completely devoid of any replicative bacilli, despite 50% of the lung tissue being used for CFU analyses. In addition to assessing the bacterial burden in random sections, we also identified and isolated individual granulomas from the two groups of animals. We observed significantly higher bacterial burdens in the granulomas of untreated animals ($P < 0.0001$) compared to 3HP-treated animals (Figure 4B). In the treated group, only three individual granulomas (out of 34 studied) or 8% harbored culturable bacilli compared to the untreated group where 32 out of 34 granulomas or 94%, harbored replicative bacilli (Figure 4B). Statistically higher bacterial burdens were also observed in extrapulmonary organs: bronchial lymph nodes ($P = 0.02$) (Figure 4C), spleen ($P = 0.01$) (Figure 4D), kidney ($P = 0.01$) (Figure 4E) and liver ($P = 0.01$) (Figure 4F). Only one out of six treated animals exhibited culturable *Mtb* in bronchial lymph nodes and spleen and none of the animals harbored bacteria in the liver or kidney.

**Pulmonary pathology in 3HP-treated and untreated macaques**

To determine the impact of 3HP treatment on the lung pathology, lung tissue was collected at necropsy and subjected to H&E staining to study the cellular and granulomatous pathology (Figure 5 and S1). The pathological findings correlated well with the clinical and microbiological findings. All six untreated control animals demonstrated granulomas in lung tissue at necropsy (Figure 5A) whereas four out of six 3HP treated macaques demonstrated no granulomas (Figure 5B) in the lung.
tissue. Detailed histopathology analysis of stereologically collected samples from all animals demonstrated robust granulomatous inflammation in the untreated group, suggestive of SIV-induced reactivation. Untreated animals demonstrated well-formed granulomas with caseous central areas (Figure 5C) and multifocal histiocytic to mixed inflammation (immature granulomas) (Figure 5D). Digital quantification of lung pathology showed significantly higher ($P = 0.02$) lung involvement (Mean of 18%, range 7% to 39%) in the untreated control group compared to 3HP treated group (Mean of 1%, range 0.28% to 2.15%) (Figure 5E). Disseminated granulomatous inflammation (bronchial lymph nodes, spleen and liver) was observed in four out of six animals in untreated group and in one out of six animals in treated group (data not shown).

**Immunologic and virologic effects of SIV infection in LTBI macaques**

SIV plasma viral loads were measured in each animal to rule out the possibility that the differences in the clinical outcomes between treated and untreated groups were due to differential viral replication (Figure 6A). No statistically significant differences were observed in the viral loads at both the acute set point and end stage of SIV infection between the two groups (Figure 6A). Flow cytometric analysis of BAL and lung cells from 3HP-treated and untreated animals that were obtained at necropsy following SIV co-infection showed that frequencies of CD4$^+$ T cells in the lungs of both groups of animals were comparable (7-9%; no statistical difference) (Figure 6B). Lung CD8$^+$ T cells were equally elevated in both groups (>75%) and were statistically indistinguishable (Figure 6B). Similarly in BAL, there was a comparable depletion of CD4$^+$ T cells in both groups with enhanced frequencies of CD8$^+$ T cells (Figure 6C).

**Discussion**

Our study demonstrates that viable *Mtb* can persist within the lungs of rhesus macaques for up to 7 months during the asymptomatic LTBI state. Further, we were able to assess the effectiveness of 3HP treatment for clearing *Mtb* in a model of LTBI and SIV-mediated reactivation. Our data show that the 3HP regimen was able to clear *Mtb* in four out of six treated macaque lungs and prevent reactivation of LTBI in all six treated animals following SIV co-infection. In comparison, all six untreated animals demonstrated clear signs of TB reactivation upon SIV co-infection. The CDC recommends 3HP as an effective treatment for LTBI in humans, and our study, shows low levels of culturable bacteria in the lungs of 3HP treated NHPs. Our study does not establish
complete sterilization of *Mtb* bacilli by 3HP, as treated animals may harbor low numbers of bacteria that are unable to cause disease in the study period. Overall, our studies establish a new animal model for evaluating the efficacy of drug regimens such as 3HP, which can be extended to study additional treatment regimens for LTBI. Moreover, this model allows for detailed immunologic and microbiological investigations in local and peripheral compartments during persistent *Mtb* infection, treatment and reactivation to TB disease.

*Mtb* is able to reside within the lung tissue in a slow or non-replicating state due to its resistance to host immunity and ability to withstand hypoxia and oxidative stress [12]. Although LTBI is associated with low-level persistence of *Mtb* without progression to disease, current diagnostics cannot detect *Mtb* in asymptomatic IGRA-positive individuals. As a result, we are unable to identify the subset of IGRA-positive and/or TST-positive individuals who harbor viable bacilli in their lungs versus those who may have cleared infection. Furthermore, studying lung-specific host immune responses associated with LTBI in humans remains challenging [13]. Our model allows for longitudinal sampling over long periods of time to monitor clinical, radiologic, pathologic, microbiologic and immunologic parameters subsequent to precise delivery of *Mtb* via aerosol route. Thus, this model provides a platform for further, more detailed investigation into immune correlates of persistence or clearance of *Mtb* infection. Analogous to IGRA-positive patients who do not develop TB disease, we found that a majority of macaques in our study remained devoid of clinical signs of TB disease following a low dose infection with *Mtb* CDC1551 [7]. Moreover, a substantial reactivation to TB disease following SIV co-infection confirmed the presence of viable *Mtb* bacilli in these animals. One of the caveats of our model is the early progression to TB disease, prior to SIV co-infection, in a minority of macaques. This is likely due to the fact that while we exposed animals to low-doses of Mtb, exposures of 10-20 cfu are still likely significantly more than the physiological exposure of most humans. Early progression to TB in our model may be analogous to individuals who progress to primary TB relatively early after infection. We believe that while the limitations of our model do not diminish the overall significance of our findings, it is nevertheless important to recognize these issues when applying this model to future studies. Characterization of LTBI in cynomolgus macaques by a heterogeneous mixture of sterile and nonsterile granulomas has also been reported [11]. It is believed that local physiology, oxygenation status, and local lung immune responses play a critical role in the balance between control of
persistent bacilli during LTBI and active replication of *Mtb* during progression to TB disease [14]. Thus, our animal model provides the important advantage of studying lung immune responses longitudinally which is difficult to study in humans [15, 16].

The CDC currently recommends the 3HP regimen as preventive treatment for LTBI in the United States and notes that the shorter 3HP regimen leads to substantially higher completion rates compared with a 9-month regimen of isoniazid alone [17]. However, the metrics for evaluating the success or failure of any treatment regimen center on epidemiologic rates of TB relapse or recurrence [18]. Using our rhesus macaque model of LTBI, we were able to directly assess 3HP-mediated clearance of persistent *Mtb* bacilli. We show that 3HP treatment significantly reduced persistent *Mtb* burdens as shown by PET/CT scans, microbiological culture and lack of LTBI reactivation upon SIV co-infection. 3HP-mediated *Mtb* clearance was also independent of differences in SIV viral loads or depletion of CD4+ T cells in BAL and lung, which were comparable in treated and untreated animals. These results suggest that the extent of pathology observed in these untreated animals resulted from recent reactivation of TB infection following SIV coinfection rather than progression of disease from the *Mtb* infection eight to nine months earlier. Drug hepatotoxicity, leading to lower rates of patient adherence is often seen during LTBI treatment. Scale up of LTBI treatment globally would be significantly impacted by reductions in the treatment duration [19]. In addition to being cost-effective and causing less hepatotoxicity, the shortened duration and frequency of 3HP dosing has resulted in much higher rates of treatment completion [20, 21]. Similar to humans, we observed no hepatotoxicity in the study animals after 3HP treatment completion. The comparative clinical trials between once-weekly 3HP and daily isoniazid for 9 months utilized percentage of patients that developed TB post-treatment as the main end-point [4, 5]. A one-month regimen of daily isoniazid-rifapentine (1HP) in HIV-infected patients living in areas of high tuberculosis prevalence was noninferior to nine months of isoniazid alone in preventing tuberculosis in this cohort [22]. Our macaque model demonstrates effective clearance of *Mtb* infection by the 3HP regimen and provides evidence that 3HP reduces persistent *Mtb* infection.

One of the limitations of our study is the inability to precisely model latently infected humans who remain asymptomatic for extended periods of time after initial exposure to *Mtb*. Rather, our model
of LTBI and its treatment more closely models recently infected contacts of TB source cases who test positive for IGRA/TST but fail to progress to TB disease within the first-year post-exposure. Given the high risk of developing TB is in the first 2 years post-exposure, recent contacts are considered to be a priority for preventive treatment [23]. Another limitation is the use of a single agent, SIV, to induce LTBI reactivation in our model. Future studies can test additional agents such as tumor necrosis factor blockade or steroid-mediated immunosuppression to induce LTBI reactivation. We were also limited by the inability to assess the impact of 3HP on nonculturable bacilli. While 3HP effectively clears culturable *Mtb*, we are unable to currently determine its impact on nonculturable bacilli.

**Conclusions**

By leveraging an NHP model of TB, we have clearly demonstrated that *Mtb* can persist in the lungs of latently infected macaques for months after infection, effectively modeling IGRA-positive contacts of TB cases in humans with LTBI. Furthermore, we provide experimental evidence of the 3HP regimen as preventive treatment for LTBI by showing that treatment with once-weekly 3HP reduced the risk of developing TB disease in the macaque-LTBI model. Together, these results confirm clinical studies on 3HP and establish a robust preclinical NHP platform for immunologic investigations of LTBI and evaluating novel drug candidates and regimens for treating contacts of drug-sensitive and drug-resistant TB cases.

**Methods**

**Animal infection and 3HP treatment**

12 naïve Indian-origin rhesus macaques were infected via aerosol with a low dose (~10 cfu) of *Mtb* CDC1551 [7, 8, 16]. Infection was confirmed by TSTs at weeks 3 and 5 post-infection. Animals were monitored for C-reactive protein (CRP), body weight and body temperature weekly. All animals were TST positive, but remained devoid of disease for up to 12 weeks, and were thus considered to have developed LTBI. They were randomly assigned to either treatment or control groups (6 animals each). The treatment group received a weekly oral dose of 15mg/kg isoniazid and 15 mg/kg rifapentine for 12 weeks beginning week 12 after aerosol infection up to week 23 post-TB infection. Oral intake was monitored by veterinary staff to ensure consumption. To confirm clearance of *Mtb* bacilli by 3HP treatment, 11 out of 12 animals were co-infected with
300 median tissue culture infectious dose SIV$_{mac239}$ intravenously at week 27 post $Mtb$ infection [7, 9]. Animals were euthanized upon signs of ATB (strong PET/CT signal, presence of culturable $Mtb$ in BAL, continuous weight-loss and high serum CRP levels and anorexia) or as time-matched controls.

**Positron emission tomography-computed tomography (PET/CT) imaging**

Longitudinal CT and PET/CT scans were performed using MEDISO’s LFER150 PET-CT scanner at 3-6 week intervals, starting from week 4 post-TB infection with the last scan prior to necropsy [24]. Briefly, we performed 18F-fluorodeoxyglucose (FDG) PET/CT scans for each anesthetized macaque using the breath-hold technique. Animals were anesthetized and intubated under supervision of a board-certified veterinarian as per approved IACUC protocols. All the animals received an intravenous injection of 5 mCi dose of 18F-FDG [25], procured from Cardinal Health radiopharmacy. The single field of view (FOV) and/or double FOV lung CT scans were performed using breath-hold as described [26]. PET scans were acquired after completion of the 40-50 min FDG uptake period. Images were visualized using Interview Fusion 3.03 (Mediso) and reconstructed using Nucline NanoScan LFER 1.07 (Mediso) with parameters as described [27]. The lung segmentation, volumetric and SUV analysis was performed using Vivoquant 4.0 (Invicro, USA) [24].

Briefly, region of interest [28] was drawn using connected thresholding referencing CT Hounsfield units (HU) for lung, and also manually to identify lung lesions as previously described [29]. Subsequently, image-derived mean standard uptake values (SUV) were calculated for the complete lung ROI and represented as total lung activity while the mean SUV for the lung lesion ROI are represented as lung lesion activity [30]. The maximum standardized uptake value (SUVmax) of the 18F-FDG in the lungs of the TB infected macaques, usually seen in the lung lesions, is represented as lung SUVmax. Granuloma count was performed by identifying and counting heterogenous TB lesions manually [31]. Animals showing numerous granulomas more than hundred and or consolidation, collapse are represented as TNTC (too numerous to count).

**Assessment of $Mtb$ infection and disease**

Weekly physical examinations including measurement of body weight, temperature and SIV viral loads were determined as previously described [7, 16, 32, 33]. Bacterial burden associated with
*Mtb* infection was determined at necropsy by plating homogenized tissue sections, as described earlier [7, 8, 16]. Individual lung lobes were sectioned into 5 µm thickness and stereologically selected for analysis that allowed for unbiased selection of lung tissue [34]. Randomly selected sections were pooled for CFU and used for histopathology. Approximately 50% of the lung tissue was pooled by lung lobe (n=5/animal), homogenized, serially diluted and plated in triplicate. Approximately 30% of the lung tissue was fixed and stained with hematoxylin and eosin using standard methods for histologic analysis and scanned with Zeiss axio scan.Z1 (Zeiss, Germany) slide scanner at 40x magnification and the digital slides were analyzed using an optimized tissue classifier in HALO v3.3 software (Indica Labs, USA). The remaining tissue was processed as single-cell suspensions for flow cytometry as described earlier [7, 16]. Bronchial lymph nodes, spleen, liver and kidney were plated for colony-forming units (CFU). All infected macaques were housed in ABSL3 facilities at the Southwest National Primate Research Center (SNPRC), where they were treated according the standards recommended by AAALAC International and the NIH guide for the Care and Use of Laboratory Animals. The study procedures were approved by the Animal Care and Use Committee of the Texas Biomedical Research Institute.

**Statistical Analysis**
Statistical analysis was performed using GraphPad Prism (version 8.4.1). A *P* value of <0.05 was considered as statistically significant. *P* < 0.05; **P* < 0.01; ***P* < 0.001; ****P* < 0.0001. Data are represented as Mean ± SEM. Specific analysis are indicated in the figure legend of each figure.

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References


Figure 1. Study outline and clinical parameters. (A) The nonhuman primate model of latent tuberculosis infection and treatment. Animals were infected with 10 cfu of *Mycobacterium tuberculosis* (*Mtb*) CDC1551, and of the 12 animals that developed latent tuberculosis infection, \( n = 6 \) were left untreated, while \( n = 6 \) were treated weekly with isoniazid and rifapentine for 3 months and rested for 1 month before coinfection with simian immunodeficiency virus (SIV). [30] Animals were monitored for signs of disease such as CRP (C-reactive protein) (B), pyrexia (C) and wasting (D) throughout the study. The thin dotted lines represent the treatment period and the thick dotted line represents SIV co-infection. (E) Survival kinetics shown as days after *Mtb* infection. (F) Blood biochemistry for serum Albumin/Globulin (A/G) (g/dL) ratio, Aspartate Aminotransferase or Serum Glutamic-Oxaloacetic Transaminase (ALT/SGOT) (units per liter of serum), Blood Urea Nitrogen/Creatinine (BUN/CREAT) (µmol/L) ratio, and Alkaline Phosphatase (ALK PHOS) (units per liter), at week 25 post-TB infection or one-week post-treatment completion for both, treated and control groups. The small dotted lines at weeks 12 and 23 mark the 3HP treatment period while the bold dotted line at week 27 marks the SIV co-infection time point.
Figure 1.
Figure 2. Computed tomography scans of (A) control and (B) 3HP-treated rhesus macaques at weeks 8-10, 22, 26 post-TB infection and at study end point. Animal ID 31438 was an active progressor and was not administered SIV. In the longitudinal CT scans performed, macaques in 3HP treatment (12 weeks, once weekly oral) group, reported resolving lung lesions as early as two to four weeks post 3HP treatment initiation (marked with black arrows), while there were no new lung lesions and preexisting lung lesions further resolved at 10 weeks post 3HP initiation (marked with black arrows).
Figure 2.
Figure 3. Positron Emission Tomography (PET) scans of treated and control rhesus macaques. (A) PET scans of 6 untreated control animals demonstrating gradual progression in TB pathology from week 26 post TB infection up to necropsy with multiple new lung lesions, increase in size of previously reported nodular lung lesions. (B) PET scans of 6 3HP- treated animals demonstrating no new lung lesions. (C) Granuloma counts, (D) Lung lesion volume, (E) Lung lesion activity, (F) Lung SUVmax and (G) total lung activity at weeks 26 and necropsy in treated and untreated controls. Data are represented as Mean+SEM. Significance was determined using Two-way ANOVA or Multiple t test using Holm-Sidak’s method, *P < 0.05; **P <0.01; ***P < 0.001.
Figure 3.
Figure 4. Bacterial persistence and burden. (A) Lung bacterial burden in animals that were left untreated for 7 months compared with animals treated with a 12-week regimen of once-weekly isoniazid and rifapentine, which mirrored results found in lung granulomas (B). Dissemination and extra thoracic bacterial burden were further measured in bronchial lymph nodes (C), spleen (D), kidney (E), and liver (F). *$P < 0.05$, and ****$P < 0.0001$ using Student’s $t$ test.
Figure 4.
Figure 5. Pulmonary pathology. Lung tissue at the time of necropsy was stereoscopically distributed for analysis by hematoxylin and eosin staining. (A) and (B) histologic analysis of lung tissues at study endpoint after SIV co-infection in untreated animals (A) and treated animals (B). (C-D) A representative image demonstrates severe pathology and bacterial burden, in multiple areas such as bronchial lumen (C), lymphangitic lesion (D) with indicated scale bars for each image. Arrowheads denote acid-fast bacilli present after Ziehl-Nielsen staining (E) Analysis of animals treated with a 12-week regimen of once-weekly isoniazid and rifapentine demonstrated significantly lower to no detectable granuloma lesions or severe consolidation prominent in coinfected animals, as shown by histologic analysis. *P < 0.05 using Student’s t test. % Pathology refers to the percentage of lung involvement in each group.
Figure 5.
Figure 6. Immune measurements. (A) Plasma viral loads after SIV infection demonstrate parallel viral infection and burden in both groups. (B and C) Analysis of CD4+ and CD8+ T cells as a percentage of CD3+ lymphocytes by flow cytometric analysis of single-cell suspensions in lung cells (B) and in BAL (C) at the time of necropsy. No significance was found using two-way ANOVA with Sidak’s correction (A) or Students’ t test (B and C). ns = not significant.
Figure 6.