Short-Course Therapy with Daily Rifapentine in a Murine Model of Latent Tuberculosis Infection

Tianyu Zhang[1], Ming Zhang[1], Ian M. Rosenthal[1,2], Jacques H. Grosset[1], and Eric L. Nuermberger[1,2]

[1]Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore; and [2]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

***Rationale*: Regimens recommended to treat latent tuberculosis infection (LTBI) are 3 to 9 months long. A 2-month rifampin+pyrazinamide regimen is no longer recommended. Shorter regimens are highly desirable. Because substituting rifapentine for rifampin in the standard regimen for active tuberculosis halves the treatment duration needed to prevent relapse in mice, we hypothesized daily rifapentine-based regimens could shorten LTBI treatment to 2 months or less.**

***Objectives*: To improve an existing model of LTBI chemotherapy and evaluate the efficacy of daily rifapentine-based regimens.**

***Methods*: Mice were immunized with a more immunogenic recombinant Bacille Calmette-Guérin strain (rBCG30) and received very low-dose aerosol infection with *Mycobacterium tuberculosis* to establish a stable lung bacterial burden below 10^4 CFU without drug treatment. Mice received a control (isoniazid alone, rifampin alone, rifampin+isoniazid, rifampin+pyrazinamide) or test (rifapentine alone, rifapentine+isoniazid, rifapentine+pyrazinamide, rifapentine+isoniazid+pyrazinamide) regimen for 8 weeks. Rifamycin doses were 10 mg/kg/d, analogous to the same human doses. Outcomes were biweekly lung CFU counts and relapse after 4 to 8 weeks of treatment.**

***Measurements and Main Results*: *M. tuberculosis* CFU counts remained stable around 3.65 $\log_{10}$ in immunized, untreated mice. Isoniazid or rifampin left all or most mice culture-positive at week 8. Rifampin+isoniazid cured 0 and 53% of mice and rifampin+pyrazinamide cured 47 and 100% of mice in 4 and 8 weeks, respectively. Rifapentine-based regimens were more active than rifampin+isoniazid and indistinguishable from rifampin+pyrazinamide.**

***Conclusions*: In this improved murine model of LTBI chemotherapy with very low lung burden, existing regimens were well represented. Daily rifapentine-based regimens were at least as active as rifampin+pyrazinamide, suggesting they could effectively treat LTBI in 6 to 8 weeks.**

Keywords: BCG; mouse; isoniazid; rifampin; pyrazinamide

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Now that a 2-month regimen of rifampin-pyrazinamide is no longer recommended to treat latent tuberculosis infection (LTBI), a new short-course regimen is highly desirable. The use of rifapentine in place of rifampin in daily treatment regimens against active tuberculosis in mice halves the duration of treatment needed for cure. Daily rifapentine-based regimens may have similar benefits in the treatment of LTBI.

What This Study Adds to the Field

This study validates an improved murine model of LTBI treatment and demonstrates that daily rifapentine-containing regimens are more effective than currently recommended LTBI regimens and may effectively treat LTBI in 6 to 8 weeks.

Identification and treatment of latent tuberculosis infection (LTBI) is necessary to eliminate tuberculosis (TB) in low-incidence countries (1–4). This strategy may soon be applied more widely now that new diagnostic tests are available to discriminate LTBI from past vaccination with Bacille Calmette-Guérin (BCG) or exposure to nontuberculous mycobacteria (5). Preventive therapy is also effective, though vastly underutilized, in reducing TB risk among HIV-infected persons with LTBI in high-prevalence settings, including persons receiving concomitant highly active antiretroviral therapy (6–9).

Current LTBI treatment guidelines generally consider isoniazid (INH) for 6 to 12 months to be the first-line regimen (1, 4, 10). Rifampin (RIF) for 4 months is an alternative in the United States and Canada (1, 4, 11). In the United Kingdom, a 3-month regimen of RIF+INH is used with success (12, 13). The combination of RIF+pyrazinamide (PZA) was first shown to have potent sterilizing activity in a model of LTBI using mice immunized with BCG (14). After clinical trials proved its efficacy among HIV-infected individuals with LTBI (15, 16), a 2-month RIF+PZA regimen was briefly in clinical use before being abandoned due to hepatotoxicity concerns (11). Based on evidence that shorter regimens are associated with higher treatment completion rates and cost savings (17–21), new short-course regimens capable of eradicating LTBI in 2 months or less are highly desirable.

Recently, a 3-month, once-weekly regimen combining INH and rifapentine (RPT), a rifamycin with a half-life (10 to 15 hours) five times longer than RIF, has shown promising efficacy in humans (22), as anticipated by studies in the murine model (23). However, the results of a massive clinical trial conducted by the Tuberculosis Trials Consortium are still awaited. We recently found that daily dosing of RPT in place of RIF in a murine model of active TB greatly increases the rifamycin exposure and halves the duration of combination chemotherapy needed to prevent relapse after treatment (24, 25). As a result, clinical trials are now underway to evaluate the efficacy and safety of daily RPT-based regimens in patients with TB. Because this beneficial effect of substituting RPT for RIF could be applicable to the treatment of LTBI as well, we hypothesized that daily administration of RPT-containing regimens could prevent relapse with 2 months or less of treatment (i.e., half the time required by the same dose of RIF).

To test our hypothesis, we used a murine model of LTBI chemotherapy that first suggested the potential of RIF+PZA and predicted the efficacy of once-weekly RPT+INH (14, 23). Although latent infection with *Mycobacterium tuberculosis*

(*Received in original form May 10, 2009; accepted in final form September 1, 2009*)

Supported by National Institutes of Health contract AI40007 and grant AI58993.

Correspondence and requests for reprints should be addressed to Eric L. Nuermberger, M.D., 1550 Orleans St., Baltimore, MD 21231-1002. E-mail: enuermb@jhmi.edu

Am J Respir Crit Care Med Vol 180. pp 1151–1157, 2009
Originally Published in Press as DOI: 10.1164/rccm.200905-0795OC on September 3, 2009
Internet address: www.atsjournals.org

cannot be precisely recapitulated in mice, this model uses immunization with BCG to enhance immune-mediated containment of *M. tuberculosis* infection and produce a stable bacterial population of limited size and multiplication rate (26). In the current study, we describe improvements to the existing model that result in stable infection with a bacillary burden ($<10^4$ CFU in the lungs) similar to or approaching the estimated burden in human LTBI and use of the model to evaluate whether daily RPT-based regimens have the potential to improve treatment of LTBI.

Some of the results of these studies have been previously reported in the form of an abstract and oral presentation (27).

METHODS

Mycobacterial Strains

M. tuberculosis H37Rv, a recombinant BCG strain overexpressing the 30-kD major secretory protein (rBCG30) (28, 29), and the Tice BCG parent strain were mouse-passaged, frozen in aliquots, and subcultured in Middlebrook 7H9 broth with 10% oleic acid-albumin-dextrose-catalase (Fisher, Pittsburgh, PA) and 0.05% Tween 80 before infection. The rBCG30 and Tice BCG strains were kind gifts from Professor Marcus A. Horwitz. The rBCG30 strain is a more efficacious vaccine than its parent in guinea pig and bovine models of pulmonary TB (28–30) and more effective than several commonly used BCG strains in guinea pigs (31).

Antimicrobials

INH, RIF, PZA, and RPT were obtained and formulated for oral administration as previously described (25). The minimum inhibitory concentrations (in μg/ml) for *M. tuberculosis* H37Rv are 0.25 for RIF, 0.06 for RPT, and 0.1 for INH on 7H11 agar and 10 for PZA on Löwenstein-Jensen medium (pH 5.5) (32).

Aerosol BCG Immunization

All animal procedures were approved by the institutional Animal Care and Use Committee. The scheme of the experiment is described in Figure 1. In brief, female, 5-week-old BALB/c mice (Charles River, Wilmington, MA) were infected with rBCG30 or Tice BCG using the Inhalation Exposure System (Glas-Col, Terre Haute, IN) and a log phase culture ($OD_{600\ nm}$, ~ 0.5). Mice were killed 1 day and 6 weeks after immunization to determine the number of bacteria implanted in the lungs and the number present at the time of challenge with *M. tuberculosis*, respectively.

Aerosol Infection with *M. tuberculosis*

Six weeks after BCG immunization, mice, including nonimmunized control animals, were infected using a 1:1,000 or 1:2,000 dilution of a 7-day-old broth culture of *M. tuberculosis* H37Rv ($OD_{600\ nm}$, 1.1). Mice were killed 1 day after infection to determine the number of bacteria implanted. Additional mice (five per group) were killed at selected time points thereafter to determine the extent of multiplication and the response to treatment.

Quantitative Culture of Lung and Spleen Homogenates

Lungs and spleens were removed aseptically and homogenized in 2.5 ml of phosphate-buffered saline using glass tissue grinders. Aliquots (0.5 ml) of undiluted homogenates and serial 10-fold dilutions were plated in parallel on (*1*) selective Middlebrook 7H11 agar (Becton-Dickinson, Franklin Lakes, NJ) enriched with 10% oleic acid-albumin-dextrose-catalase; (*2*) 7H11 agar supplemented with 40 μg/ml of hygromycin (Roche Diagnostics, Indianapolis, IN) to select for rBCG30, which has a hygromycin resistance marker; and (*3*) 7H11 agar supplemented with 4 μg/ml of 2-thiophenecarboxylic acid hydrazide (TCH) (Sigma, St. Louis, MO) to select for *M. tuberculosis* (14). Plates were incubated for 28 days at 37°C in a 5% CO_2 environment before final CFU counts were determined. CFU counts reported for rBCG30 and *M. tuberculosis* are the counts determined on hygromycin-containing and TCH-containing plates, respectively.

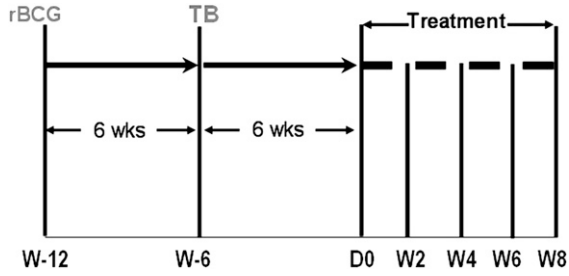

Figure 1. Scheme of the treatment efficacy experiment. D0 = initiation of treatment; rBCG = immunization with rBCG30 12 weeks before treatment (W−12); TB = low-dose challenge with *Mycobacterium tuberculosis* 6 weeks before treatment (W−6). End points were lung colony-forming unit counts at Week 2 (W2), Week 4 (W4), Week 6 (W6), and Week 8 (W8) of treatment and culture-positive relapse rates assessed 3 months after completing treatment for 4, 6, and 8 weeks.

Experiment Comparing the Efficacy of Tice BCG and rBCG30 as Immunizing Agents

Mice were immunized with rBCG30 or Tice BCG and then infected with *M. tuberculosis* as described above. Nonimmunized mice infected with *M. tuberculosis* served as controls. Organ CFU counts were determined the day after BCG immunization and 1 day and 6 and 10 weeks after *M. tuberculosis* infection.

Experiment Comparing the Efficacy of RPT-based Regimens with That of Standard LTBI Regimens

The scheme for the treatment efficacy experiment is presented in Figure 1. Mice were immunized with rBCG30 and then infected with *M. tuberculosis* as described above. After *M. tuberculosis* infection, rBCG30-immunized mice from each infection run (four runs in all) were block-randomized by run to one of the following groups (Table 1): untreated, INH, RIF, RIF+INH, RIF+PZA, RPT, RPT+INH, RPT+PZA, and RPT+INH+PZA. Treatment began 6 weeks after challenge (on Day 0), with all drugs administered by gavage, 5 d/wk. The drug doses (in mg/kg) were: INH (10), RIF (10), PZA (150), and RPT (10). These doses of INH, RIF, and PZA each produce an area under the serum concentration-time curve in mice that is similar to the area under the curve observed in humans receiving recommended doses (33). RPT 10 mg/kg in mice is equivalent to 10 mg/kg (600 mg) in humans (25).

Efficacy was assessed on the bases of lung CFU counts and the proportions of mice with culture-positive relapse after treatment completion. CFU counts were performed after 2, 4, 6, and 8 weeks of treatment. The proportion of culture-positive relapses was determined by holding cohorts of 15 mice for three additional months after completing 4, 6, and 8 weeks of treatment, then killing the mice to determine the proportion with positive lung cultures, as defined by at least 1 CFU of *M. tuberculosis* detected after plating each entire lung homogenate onto five 7H11 plain plates with 0.5 ml per plate. Colonies were confirmed as *M. tuberculosis* or rBCG30 by replating on TCH- or hygromycin-containing plates, respectively. Only mice receiving RPT-containing regimens were assessed at the 6-week time point.

The use of five mice per group for CFU counts provided greater than 80% power to detect 0.5 $\log_{10}$ differences when we retrospectively examined the results of similar past experiments. The use of 15 mice per group for relapse assessment provides greater than 80% power to detect 40 percentage point differences in the relapse rate, after setting α at 0.01 to adjust for five simultaneous comparisons. Smaller differences are unlikely to be meaningful in terms of shortening the duration of treatment.

Statistical Analysis

CFU counts (x) were log-transformed as ($x + 1$) before analysis. Group means were compared by unpaired t tests (untreated BCG immunized versus nonimmunized mice) or one-way analysis of variance with Dunnett's posttest (all other comparisons) using GraphPad Prism version 4.01 (GraphPad, San Diego, CA). Group relapse proportions

TABLE 1. *MYCOBACTERIUM TUBERCULOSIS* COLONY-FORMING UNIT COUNTS DURING TREATMENT

Regimen	D0 Lung $\log_{10}$ CFU	Mean lung $\log_{10}$ CFU			
		Week 2	Week 4	Week 6	Week 8
Untreated	3.65 ± 0.30*	3.61 ± 0.11	3.71 ± 0.56	nd	3.64 ± 0.51
INH		3.01 ± 0.26	2.61 ± 0.44	nd	1.71 ± 0.54
RIF		2.90 ± 0.38	1.87 ± 0.49	nd	0.33 ± 0.30†
RIF+INH		2.17 ± 0.46	1.08 ± 0.47	nd	0
RIF+PZA		1.87 ± 0.42	0.18 ± 0.15†	nd	0
RPT		2.50 ± 0.36	0.67 ± 0.15	0	nd
RPT+INH		1.25 ± 0.44	0.19 ± 0.43‡	0	nd
RPT+PZA		0.88 ± 0.13	0.13 ± 0.15‡	0	nd
RPT+INH+PZA		1.27 ± 0.40	0	0	nd

Definition of abbreviations: CFU = colony-forming unit; D0 = start of treatment; INH = isoniazid; nd, not done; PZA = pyrazinamide; RIF = rifampin; RPT = rifapentine.

* Values are mean ± SD.

† Three of five mice were culture positive.

‡ One of five mice was culture positive.

were compared using Fisher's exact test, adjusting for multiple comparisons, using STATA 8.2 (StataCorp, College Station, TX).

RESULTS

Experiment Comparing the Efficacy of Tice BCG and rBCG30 as Immunizing Agents

Mice were aerosol-infected with rBCG30 or Tice BCG. Mean (± SD) lung BCG CFU counts on the day after immunization were 3.61 ± 0.07 and 3.03 ± 0.24 $\log_{10}$, respectively. Six weeks later, at the time of challenge with *M. tuberculosis*, the mean lung BCG CFU counts had increased to 5.86 ± 0.18 and 4.82 ± 0.30, respectively. Six and ten weeks after challenge, the mean lung rBCG30 CFU counts were 4.39 ± 0.01 and 3.77 ± 0.17, respectively, and the mean spleen rBCG30 CFU counts were 4.11 ± 0.16 and 4.42 ± 0.26, respectively. Tice BCG CFU counts could not be determined at these time points due to lack of an effective selection marker to differentiate Tice BCG from *M. tuberculosis*.

On the day after challenge, the mean lung *M. tuberculosis* CFU count was 1.36 ± 0.03. Six weeks later, at the time treatment ordinarily begins in this model, the mean lung *M. tuberculosis* CFU counts were 6.21 ± 0.14, 4.88 ± 0.33, and approximately 4.4 (precise figure not available due to a technical problem with some dilutions) in nonimmunized, Tice BCG-immunized, and rBCG30-immunized mice, respectively (Figure 2A). Ten weeks after challenge, the mean lung *M. tuberculosis* CFU counts were 5.92 ± 0.15, 4.73 ± 0.24, and 4.14 ± 0.29 in nonimmunized, Tice BCG-immunized, and rBCG30-immunized mice, respectively. Spleen CFU counts showed a similar pattern. Six weeks after challenge, the mean spleen *M. tuberculosis* CFU counts were 4.94 ± 0.18, 3.68 ± 0.77, and 2.08 ± 0.86 in nonimmunized, Tice BCG-immunized, and rBCG30-immunized mice, respectively (Figure 2B). Only two of five rBCG30-immunized mice were culture positive (lower limit of detection, 5 CFU). Ten weeks after challenge, the mean spleen *M. tuberculosis* CFU counts were 5.01 ± 0.21, 3.92 ± 0.41, and 2.91 ± 0.62 in nonimmunized, Tice BCG–immunized, and rBCG30-immunized mice, respectively. The better containment of *M. tuberculosis* growth after immunization with rBCG30 during this preliminary experiment led us to use rBCG30 for the efficacy experiment.

rBCG30 CFU Counts during the Treatment Efficacy Experiment

The day after aerosol rBCG30 immunization (W-12), the mean (± SD) lung rBCG30 CFU count was 3.71 ± 0.04 $\log_{10}$ per lung. Six weeks later, on the day of infection with *M. tuberculosis* (W−6), the mean rBCG30 lung CFU count had increased to 6.14 ± 0.15 $\log_{10}$. Six weeks after infection with *M. tuberculosis*, at the start of treatment (D0), the mean rBCG30 lung CFU count had fallen to 4.35 ± 0.10. During the treatment period,

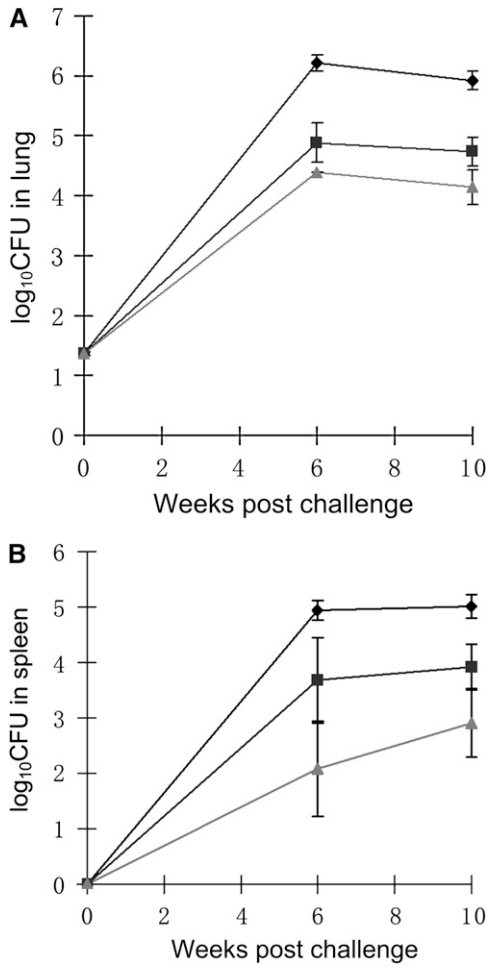

Figure 2. *M. tuberculosis* CFU counts in (*A*) lungs and (*B*) spleens after low-dose aerosol infection with *M. tuberculosis* in nonimmunized, Bacille Calmette-Guérin (BCG) Tice-immunized, and rBCG30-immunized mice in lungs. *Diamonds* = nonimmunized; *squares* = Tice BCG-immunized; *triangles* = rBCG30-immunized.

the mean CFU counts of rBCG30 remained stable in untreated animals, with values of 3.75 ± 0.70, 4.12 ± 0.28, and 3.70 ± 0.55 at W2, W4, and W8, respectively. Treatment with INH steadily reduced the rBCG30 CFU counts to 3.50 ± 0.23, 3.03 ± 0.11, and 1.97 ± 0.12 at W2, W4, and W8, respectively. Rifamycin-containing regimens were much more active than INH against rBCG30. By W4, rBCG30 was undetectable in all rifamycin-treated mice except two of five mice in the RIF+INH and RIF+PZA groups. No rBCG30 was detected in RPT-treated mice at W6 or in RIF-treated mice at W8.

M. tuberculosis CFU Counts during the Treatment Efficacy Experiment

At W−6, mice were aerosol-infected with *M. tuberculosis*. The following day, mean lung *M. tuberculosis* CFU counts were 1.03 ± 0.20 and 1.13 ± 0.19 (or approximately 11–14 CFU) in nonimmunized and rBCG30-immunized mice, respectively. Six weeks later, at D0, the mean lung *M. tuberculosis* CFU count was 6.01 ± 0.19 $\log_{10}$ in nonimmunized mice and only 3.73 ± 0.15, 3.86 ± 0.16, 3.60 ± 0.40, and 3.40 ± 0.31 in immunized mice infected in runs 1 through 4, respectively, for an overall mean of 3.65 ± 0.30. The mean lung *M. tuberculosis* CFU count in rBCG30-immunized, untreated mice remained stable below 4 $\log_{10}$ for the duration of the 8-week treatment period (Table 1).

All regimens displayed bactericidal activity but differed in their potency. INH was the least effective regimen. All INH-treated mice remained culture-positive for *M. tuberculosis* at W8 with a mean CFU count of 1.71 ± 0.56 $\log_{10}$. RIF was more effective, leaving only three of five mice culture-positive for *M. tuberculosis* at W8 with 1, 1, and 3 CFU per lung, respectively. The combination RIF+INH was more effective than either drug alone and rendered all mice culture negative between W4 and W8. RIF+PZA was the most active control regimen, leaving only three of five mice culture positive for *M. tuberculosis* with 1 CFU per lung at W4 and rendering all five mice culture negative at W8.

Each RPT-containing regimen was significantly more active than the corresponding RIF-containing control regimen, although the difference in *M. tuberculosis* lung CFU counts between the RIF+PZA and RPT+PZA groups narrowly missed statistical significance ($P = 0.06$) after adjustment for multiple comparisons. RPT alone rendered all mice culture negative at W6. RPT+INH was more effective than RPT alone at W2 and rendered four of five mice culture negative at W4 and all mice negative at W6. RPT+PZA and RPT+INH+PZA had a similar effect, although the latter regimen rendered all five mice culture negative at W4.

Relapse Assessment after Treatment Completion

The proportions of mice with positive *M. tuberculosis* lung cultures 3 months after treatment completion are presented in Table 2. As expected, all 15 mice receiving just 4 weeks of RIF+INH were culture positive at follow-up. However, after 8 weeks of treatment, only 47% relapsed. Treatment with RIF+PZA for 4 and 8 weeks resulted in 53 and 0% relapse, respectively. Compared with RIF+INH, all other regimens were more effective at preventing relapse after 4 and/or 8 weeks of treatment. Results obtained with RPT-containing regimens were indistinguishable from results obtained with RIF+PZA despite the fact that all RPT-containing combinations resulted in lower CFU counts at 2 weeks compared with RIF+PZA. After 4 weeks of treatment, the proportion of mice relapsing was higher among RPT+INH-treated mice than among RPT-treated mice, although the difference was not statistically significant after adjusting for multiple comparisons. In addition, no significant differences were observed in the CFU counts and the time to stable cure without any relapse was 8 weeks with both regimens, suggesting that the difference in relapse at 4 weeks was due to random chance rather than to antagonism between RPT and INH.

TABLE 2. RESULTS OF RELAPSE ASSESSMENT

Treatment Group	Percentage (proportion) with Positive *M. tuberculosis* Cultures 3 mo after Completing Treatment		
	4 wk	6 wk	8 wk
RIF+INH	100 (15/15)	nd	47 (7/15)
RIF+PZA	53 (8/15)*	nd	0 (0/15)*
RPT	40 (6/15)*	7 (1/15)	0 (0/15)*
RPT+INH	86 (12/14)	14 (2/14)	0 (0/15)*
RPT+PZA	47 (7/15)*	0 (0/15)	nd
RPT+INH+PZA	40 (6/15)*	7 (1/15)	nd

Definition of abbreviations: INH = isoniazid; nd = not done; PZA = pyrazinamide; RIF = rifampin; RPT = rifapentine.

* $P < 0.05$ vs. RIF+INH after adjustment for multiple comparisons.

Despite replating recovered colonies on hygromycin-containing media, no rBCG30 colonies were found among the mice relapsing after 4 weeks of RIF+INH or RIF+PZA. rBCG30 was found to be the sole identifiable cause of relapse in 1, 3, and 1 mice receiving 4 weeks of RPT, RPT+PZA, and RPT+INH+PZA, respectively. These relapses are not reported in Table 2. No rBCG30 was found in any mouse relapsing after 6 or 8 weeks of treatment.

DISCUSSION

In this study, we used an improved paucibacillary model of *M. tuberculosis* infection in mice to compare daily RPT-containing regimens with several control regimens known to be effective against LTBI. As hypothesized, daily RPT alone and RPT+INH were more active than currently recommended regimens for LTBI, including INH alone, RIF alone, and RIF+INH, and were as effective as RIF+PZA. The combinations of RPT+PZA and RPT+INH+PZA were no more effective than RIF+PZA, despite the fact that substitution of RPT for RIF in a murine model of active TB shortens the duration of treatment necessary for cure from 6 to 3 months or less (24, 25).

Human LTBI is characterized by a small population of nonactively multiplying bacteria actively contained by host defenses. Because 10^4 CFU/ml is the lower limit of detection with acid-fast smears and latent lesions found at autopsy may be culture positive but smear negative (34), the bacillary burden in LTBI is assumed to be 10^4 or fewer CFU (26). Infection of naive mice with virulent *M. tuberculosis* is not contained as readily. Even low-dose aerosol or intratracheal infection with 50 to 1,000 CFU results in a bacillary burden of approximately 10^5 to 10^6 CFU in the lungs (35–38). Moreover, unlike LTBI in humans, infected mice that go untreated succumb to the disease. Two basic models of LTBI in mice may be considered for the purpose of chemotherapy research. In the so-called "Cornell model," mice are infected with *M. tuberculosis* and treated with INH and high-dose PZA long enough to produce a state in which a proportion of bacilli is viable but noncultivable under standard culture conditions (39). When left without treatment, these "persisters" resume normal growth in the mice, become cultivable, and cause a relapse. This presents the opportunity to demonstrate that a drug or drug regimen can kill the persisters and prevent relapse. Despite its appeal, the Cornell model has several disadvantages as a model for the chemotherapy of LTBI. First, the required pretreatment with INH+PZA may produce a bacterial state fundamentally different from that

occurring in human LTBI, in which bacterial growth is arrested and subsequently prevented by host immune mechanisms. Moreover, the model is technically demanding, and the proportion of mice relapsing is variable and highly dependent on the experimental conditions, requiring large numbers of mice for adequate statistical power (40). To our knowledge, only a single study has compared more than one existing LTBI regimen in the Cornell model. That study found no convincing differences between the activity of RIF alone, RIF+INH, RIF+PZA, and RIF-INH-PZA (41).

A second approach to model LTBI in mice is to use a low-dose aerosol or intratracheal infection, as described in the preceding paragraph. Although the infection is arrested by acquired host immunity (42), the bacterial burden is higher than that thought to be present in LTBI. Furthermore, we are not aware of any study comparing the efficacy of LTBI regimens in the low-dose aerosol model.

The model used in the current study may be considered as a variation on the low-dose aerosol model in which the specific antimycobacterial immune response of the mice is augmented by immunization with BCG to obtain a smaller, nonactively multiplying population of tubercle bacilli that is therefore closer to the bacterial burden of human LTBI (26). Use of the RIF+PZA combination as a short-course treatment for LTBI was first proposed two decades ago after demonstration of its potent activity in a similar model (14). The model has since been refined by changing to the aerosol route of delivery for BCG, which is a more effective route of immunization against an aerosol *M. tuberculosis* challenge (26). For the current study, the model was further improved by using a stronger immunizing agent, rBCG30, and a lower *M. tuberculosis* challenge dose to produce a stable lung infection with 10^4 or fewer CFU of *M. tuberculosis*, which persists for at least 8 weeks in the absence of treatment. Although mice are not considered to develop LTBI, the clinical relevance of this model for recapitulating the chemotherapy of human LTBI is well demonstrated by the comparative activity of existing LTBI regimens in this study. The ranking of these regimens in order of increasing activity in this model (i.e., INH < RIF < RIF+INH < RIF+PZA) is consistent with the decreasing treatment durations recommended when the same regimens are used to treat LTBI in humans (1, 12). Moreover, the duration of treatment necessary to cure all mice was 2 months with RIF+PZA, consistent with the efficacy of the same treatment duration in clinical trials (15, 16). RIF+INH was less effective but still cured over half of the mice by 2 months and may be expected to cure most, if not all, by 3 months, consistent with its clinical usage (12, 13, 43). RIF alone was less effective than RIF+INH but rendered some mice culture negative by 2 months, suggesting that 4 months may be sufficient to cure many animals. INH alone was least effective and would have likely required more than 4 months given its limited sterilizing activity in similar experiments performed in the past (14, 23). Although other mouse models, such as the Cornell model and low-dose aerosol infection models, have been used to test treatments against LTBI (41, 44–47), no model has been validated as a model of LTBI chemotherapy in this way. This evidence base supports use of the BCG-immunized paucibacillary mouse model in future studies of the potential contribution of new agents to treatment of LTBI.

In this study, RPT alone and RPT+INH were as efficacious as RIF+PZA, curing nearly all tested mice by 6 weeks and all tested mice by 8 weeks. Given the past track record of the model and the human-like pharmacokinetics of RIF and RPT in the mouse (25, 48), these results raise hopes that, if safe and tolerable in humans, daily RPT administration could provide new regimens capable of curing LTBI in 6 to 8 weeks. RPT+PZA was included for comparison with RIF+PZA knowing that it is not likely to be a feasible regimen due to the same hepatotoxicity concerns that led to withdrawal of RIF+PZA from treatment guidelines (11). However, it is surprising that RPT+PZA and RPT+INH+PZA were not more effective than RIF+PZA and were unable to cure the infection in less than 6 to 8 weeks. This is despite the fact that the RPT+INH+PZA regimen is capable of curing mice in just 10 to 12 weeks in an active TB model, when bacterial burdens at the start of treatment are as much as four orders of magnitude higher than in the current experiment and the standard RIF+INH+PZA regimen requires 20 to 24 weeks to prevent relapse (24, 25). That the limited bacillary burden in this study could not be eliminated in less than 6 to 8 weeks is evidence that the immunization procedure resulted in a population highly enriched with "persister" bacteria with phenotypic tolerance to the bactericidal effect of TB drugs. Indeed, in recently published studies of RPT+INH+PZA in an active TB model (24, 25), the initial 4 weeks of treatment reduced the initial lung burden from 7.21 to 7.45 $\log_{10}$ CFU to 3.58 to 4.13 $\log_{10}$ CFU, leaving a lung burden of persisters like that present at the start of treatment in the current study. From this point on, an additional 6 to 8 weeks of treatment was required in either model to prevent relapse after treatment completion. These comparisons support speculation that the phenotypic state of latency resulting from immune containment is not markedly dissimilar from the phenotypic state of persistence revealed by treatment of active TB. The finding that RPT+PZA and RPT+INH+PZA do not eliminate these persisters any faster than RIF+PZA may indicate that further increases in rifamycin exposure may be incapable of shortening the duration of treatment much more than 6 to 8 weeks, as observed here. Indeed, in the active TB model, we have observed that the combination of RPT+PZA plus moxifloxacin is unable to cure all mice in 4 weeks, even when the RPT dose is increased 16-fold to 160 mg/kg/d, despite documented dose-linear increases in drug exposure (49). Therefore, there may exist a biological barrier to the rate at which persisters can be eliminated with existing drugs. If so, new drugs with new mechanisms of action will likely be necessary to further shorten the duration of treatment.

Even if such a barrier exists, it remains clear that significant reductions in the duration of treatment necessary to cure active TB and LTBI may be attainable by increasing rifamycin exposures beyond what is obtained with currently recommended doses of rifampin (24, 25, 48). Based on the current study, daily treatment with RPT at 10 mg/kg/d or RPT+INH may be capable of eradicating LTBI in 6 to 8 weeks. At least three Phase II clinical trials of daily RPT-containing regimens for active TB disease are planned or underway. If such regimens are shown to be more effective than the standard RIF-containing regimen while remaining safe and tolerable, daily RPT-containing regimens deserve serious consideration as abbreviated treatment regimens for LTBI.

In conclusion, we present an improved paucibacillary model of murine TB in which the response to treatment with clinically proven LTBI regimens mirrors that observed in humans. When evaluated in this model, daily RPT-containing regimens prevent relapse after 6 to 8 weeks of treatment and are at least as effective as RIF+PZA. Although the efficacy and safety of such regimens should be confirmed in human trials before their clinical use is recommended, the present results suggest that daily RPT-containing regimens may provide for the return of highly efficacious short-course regimens for LTBI.

Conflict of Interest Statement: T.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.Z.

does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. I.M.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.H.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.L.N. received $5,001–$10,000 for promotional and nonpromotional lectures from Pfizer, $5,001–$10,000 for promotional lectures from Wyeth, $5,001–$10,000 for promotional and nonpromotional lectures from Abbott, more than $100,001 from Otsuka Pharmaceuticals, more than $100,001 from Pfizer in industry-sponsored grants, a pending patent for Combination treatment for tuberculosis from Pfizer, and holds $5,001–$10,000 from Wyeth, $1,001–$5,000 from Viropharma, $1,001–$5,000 from Mylan, and $1,001–$5,000 from Amgen in stock ownership or options.

References

1. Centers for Disease Control and Prevention and American Thoracic Society. Targeted tuberculin testing and treatment of latent tuberculosis infection. *MMWR Morb Mortal Wkly Rep* 2000;49:1–51.
2. Broekmans JF, Migliori GB, Rieder HL, Lees J, Ruutu P, Loddenkemper R, Raviglione MC, World Health Organization. European framework for tuberculosis control and elimination in countries with a low incidence: recommendations of the World Health Organization (WHO), International Union Against Tuberculosis and Lung Disease (IUATLD) and Royal Netherlands Tuberculosis Association (KNCV) Working Group. *Eur Respir J* 2002;19:765–775.
3. Mack U, Migliori GB, Sester M, Rieder HL, Ehlers S, Goletti D, Bossink A, Magdorf K, Holscher C, Kampmann B, *et al.* LTBI: latent tuberculosis infection or lasting immune responses to *M. tuberculosis*? A TBNET consensus statement. *Eur Respir J* 2009;33:956–973.
4. Public Health Agency of Canada and Canadian Lung Association/ Canadian Thoracic Association. Canadian tuberculosis standards, 6th ed. Ottowa: Ministry of Health; 2007. Cat no. HP40-18/2007E.
5. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008;149:177–184.
6. Golub JE, Pronyk P, Mohapi L, Thsabangu N, Moshabela M, Struthers H, Gray GE, McIntyre JA, Chaisson RE, Martinson NA. Isoniazid preventive therapy, HAART and tuberculosis risk in HIV-infected adults in South Africa: a prospective cohort. *AIDS* 2009;23:631–636.
7. Golub JE, Saraceni V, Cavalcante SC, Pacheco AG, Moulton LH, King BS, Efron A, Moore RD, Chaisson RE, Durovni B. The impact of antiretroviral therapy and isoniazid preventive therapy on tuberculosis incidence in HIV-infected patients in Rio De Janeiro, Brazil. *AIDS* 2007;21:1441–1448.
8. Woldehanna S, Volmink J. Treatment of latent tuberculosis infection in HIV infected persons. *Cochrane Database Syst Rev* 2004;CD000171.
9. World Health Organization. Global tuberculosis control: epidemiology, planning, financing: WHO Report 2009. Geneva: World Health Organization; 2009. WHO/HTM/TB/2009.411.
10. British Thoracic Society. Control and prevention of tuberculosis in the UK: code of practice 2000. Joint Tuberculosis Committee of the British Thoracic Society. *Thorax* 2000;55:887–901.
11. Centers for Disease Control and Prevention. Update: adverse event data and revised American Thoracic Society/CDC recommendations against the use of rifampin and pyrazinamide for treatment of latent tuberculosis infection–United States, 2003. *MMWR Morb Mortal Wkly Rep* 2003;52:735–739.
12. Joint Tuberculosis Committee of the British Thoracic Society. Chemotherapy and management of tuberculosis in the UK: recommendations 1998. *Thorax* 1998;53:536–548.
13. Spyridis NP, Spyridis PG, Gelesme A, Sypsa V, Valianatou M, Metsou F, Gourgiotis D, Tsolia MN. The effectiveness of a 9-month regimen of isoniazid alone versus 3- and 4-month regimens of isoniazid plus rifampin for treatment of latent tuberculosis infection in children: results of an 11-year randomized study. *Clin Infect Dis* 2007;45:715–722.
14. Lecoeur HF, Truffot-Pernot C, Grosset JH. Experimental short-course preventive therapy of tuberculosis with rifampin and pyrazinamide. *Am Rev Respir Dis* 1989;140:1189–1193.
15. Gordin F, Chaisson RE, Matts JP, Miller C, de Lourdes GM, Hafner R, Valdespino JL, Coberly J, Schechter M, Klukowicz AJ, *et al.* Rifampin and pyrazinamide vs isoniazid for prevention of tuberculosis in HIV-infected persons: an international randomized trial. *JAMA* 2000; 283:1445–1450.
16. Halsey NA, Coberly JS, Desormeaux J, Losikoff P, Atkinson J, Moulton LH, Contave M, Johnson M, Davis H, Geiter L, *et al.* Randomised trial of isoniazid versus rifampicin and pyrazinamide for prevention of tuberculosis in HIV-1 infection. *Lancet* 1998;351:786–792.
17. Cook PP, Maldonado RA, Yarnell CT, Holbert D. Safety and completion rate of short-course therapy for treatment of latent tuberculosis infection. *Clin Infect Dis* 2006;43:271–275.
18. Holland DP, Sanders GD, Hamilton CD, Stout JE. Costs and cost effectiveness of four treatment regimens for latent tuberculosis infection. *Am J Respir Crit Care Med* 2009;179:1055–1060.
19. Lardizabal A, Passannante M, Kojakali F, Hayden C, Reichman LB. Enhancement of treatment completion for latent tuberculosis infection with 4 months of rifampin. *Chest* 2006;130:1712–1717.
20. Menzies D, Long R, Trajman A, Dion MJ, Yang J, Al Jahdali H, Memish Z, Khan K, Gardam M, Hoeppner V, *et al.* Adverse events with 4 months of rifampin therapy or 9 months of isoniazid therapy for latent tuberculosis infection: a randomized trial. *Ann Intern Med* 2008;149:689–697.
21. Page KR, Sifakis F, Montes de Oca R, Cronin WA, Doherty MC, Federline L, Bur S, Walsh T, Karney W, Milman J, *et al.* Improved adherence and less toxicity with rifampin vs isoniazid for treatment of latent tuberculosis: a retrospective study. *Arch Intern Med* 2006;166: 1863–1870.
22. Schechter M, Zajdenverg R, Falco G, Barnes GL, Faulhaber JC, Coberly JS, Moore RD, Chaisson RE. Weekly rifapentine/isoniazid or daily rifampin/pyrazinamide for latent tuberculosis in household contacts. *Am J Respir Crit Care Med* 2006;173:922–926.
23. Nuermberger E, Tyagi S, Williams KN, Rosenthal I, Bishai WR, Grosset JH. Rifapentine, moxifloxacin, or DNA vaccine improves treatment of latent tuberculosis in a mouse model. *Am J Respir Crit Care Med* 2005;172:1452–1456.
24. Rosenthal IM, Zhang M, Almeida D, Grosset JH, Nuermberger EL. Isoniazid or moxifloxacin in rifapentine-based regimens for experimental tuberculosis? *Am J Respir Crit Care Med* 2008;178:989–993.
25. Rosenthal IM, Zhang M, Williams KN, Peloquin CA, Tyagi S, Vernon AA, Bishai WR, Chaisson RE, Grosset JH, Nuermberger EL. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. *PLoS Med* 2007;4:e344.
26. Nuermberger EL, Yoshimatsu T, Tyagi S, Bishai WR, Grosset JH. Paucibacillary tuberculosis in mice after prior aerosol immunization with *Mycobacterium bovis* BCG. *Infect Immun* 2004;72:1065–1071.
27. Zhang T, Zhang M, Grosset J, Nuermberger E. Rifapentine-based regimens cure latent tuberculosis infection (LTBI) in 2 months or less in a mouse model [abstract]. *Am J Respir Crit Care Med* 2009;179: A1017.
28. Horwitz MA, Harth G. A new vaccine against tuberculosis affords greater survival after challenge than the current vaccine in the guinea pig model of pulmonary tuberculosis. *Infect Immun* 2003;71:1672–1679.
29. Horwitz MA. Recombinant BCG expressing *Mycobacterium tuberculosis* major extracellular proteins. *Microbes Infect* 2005;7:947–954.
30. Horwitz MA, Harth G, Dillon BJ, Maslesa-Galic S. A novel live recombinant mycobacterial vaccine against bovine tuberculosis more potent than BCG. *Vaccine* 2006;24:1593–1600.
31. Horwitz MA, Harth G, Dillon BJ, Maslesa-Galić S. Commonly administered BCG strains including an evolutionarily early strain and evolutionarily late strains of disparate genealogy induce comparable protective immunity against tuberculosis. *Vaccine* 2009;27:441–445.
32. Grosset J, Lounis N, Truffot-Pernot C, O'Brien RJ, Raviglione MC, Ji B. Once-weekly rifapentine-containing regimens for treatment of tuberculosis in mice. *Am J Respir Crit Care Med* 1998;157:1436–1440.
33. Grosset J, Truffot-Pernot C, Lacroix C, Ji B. Antagonism between isoniazid and the combination pyrazinamide-rifampin against tuberculosis infection in mice. *Antimicrob Agents Chemother* 1992;36:548–551.
34. Opie EJ, Aronson JD. Tubercle bacilli in latent tuberculous lesions and in lung tissue without tuberculous lesions. *Arch Pathol Lab Med* 1927; 4:1–21.
35. Brooks JV, Orme IM. Evaluation of once-weekly therapy for tuberculosis using isoniazid plus rifamycins in the mouse aerosol infection model. *Antimicrob Agents Chemother* 1998;42:3047–3048.
36. Kelly BP, Furney SK, Jessen MT, Orme IM. Low-dose aerosol infection model for testing drugs for efficacy against *Mycobacterium tuberculosis. Antimicrob Agents Chemother* 1996;40:2809–2812.
37. Arriaga AK, Orozco EH, Aguilar LD, Rook GA, Hernandez PR. Immunological and pathological comparative analysis between experimental latent tuberculous infection and progressive pulmonary tuberculosis. *Clin Exp Immunol* 2002;128:229–237.

38. Rhoades ER, Frank AA, Orme IM. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent *Mycobacterium tuberculosis*. *Tuber Lung Dis* 1997;78:57–66.
39. McCune RM, Tompsett R. Fate of *Mycobacterium tuberculosis* in mouse tissues as determined by the microbial enumeration technique. *J Exp Med* 1956;104:737–802.
40. Lenaerts AJ, Chapman PL, Orme IM. Statistical limitations to the Cornell Model of latent tuberculosis infection for the study of relapse rates. *Tuberculosis (Edinb)* 2004;84:361–364.
41. Dhillon J, Dickinson JM, Sole K, Mitchison DA. Preventive chemotherapy of tuberculosis in Cornell model mice with combinations of rifampin, isoniazid, and pyrazinamide. *Antimicrob Agents Chemother* 1996;40:552–555.
42. Lazarevic V, Nolt D, Flynn JL. Long-term control of mycobacterium tuberculosis infection is mediated by dynamic immune responses. *J Immunol* 2005;175:1107–1117.
43. Ormerod LP. Rifampicin and isoniazid prophylactic chemotherapy for tuberculosis. *Arch Dis Child* 1998;78:169–171.
44. Dhillon J, Allen BW, Hu YM, Coates AR, Mitchison DA. Metronidazole has no antibacterial effect in Cornell model murine tuberculosis. *Int J Tuberc Lung Dis* 1998;2:736–742.
45. Miyazaki E, Chaisson RE, Bishai WR. Analysis of rifapentine for preventive therapy in the Cornell mouse model of latent tuberculosis. *Antimicrob Agents Chemother* 1999;43:2126–2130.
46. Scanga CA, Mohan VP, Joseph H, Yu K, Chan J, Flynn JL. Reactivation of latent tuberculosis: variations on the Cornell murine model. *Infect Immun* 1999;67:4531–4538.
47. Brooks JV, Furney SK, Orme IM. Metronidazole therapy in mice infected with tuberculosis. *Antimicrob Agents Chemother* 1999;43:1285–1288.
48. Rosenthal IM, Williams K, Tyagi S, Peloquin CA, Vernon AA, Bishai WR, Grosset JH, Nuermberger EL. Potent twice-weekly rifapentine-containing regimens in murine tuberculosis. *Am J Respir Crit Care Med* 2006;174:94–101.
49. Rosenthal IM, Zhang M, Grosset JH, Nuermberger EL. Rifapentine-containing regimens cure murine tuberculosis in weeks rather than months [abstract]. *Am J Respir Crit Care Med* 2008;177:A789.