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An adjunctive therapeutic vaccine against reactivation and post-treatment relapse tuberculosis

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Abstract

Preventing latently infected or inadequately treated individuals from progressing to active disease could make a major impact on tuberculosis (TB) control worldwide. The purpose of this study was to evaluate a new approach to prevent reactivation and TB relapse that combines drug treatment and vaccination. *Mycobacterium tuberculosis* harbors a gene called *mce1R* that, in vivo, negatively regulates a 13-gene cluster called the *mce1* operon. In a Cornell mouse model, BALB/c mice infected with *M. tuberculosis* H37Rv disrupted in *mce1R* consistently develop latent infection and reactivation disease. We used this new mouse model to test a recombinant *M. tuberculosis* cell wall protein (Mce1A), encoded by a gene in the *mce1* operon, for its ability to prevent post-treatment TB. At 32 weeks of follow-up, a complete sterilizing protection was observed in lungs of the vaccinated mice. Mce1A but not phosphate-buffered saline administered intraperitoneally during the period of latent infection prevented disease progression and proliferation of *M. tuberculosis* *mce1R* mutant. The only visible lung lesions in vaccinated mice included small clusters of lymphocytes, while the unvaccinated mice showed progressively enlarging granulomas comprised of foamy macrophages surrounded by lymphocytes. The combination of anti-TB drugs and a vaccine may serve as a powerful treatment modality against TB reactivation and relapse.

Keywords

tuberculosis; latent TB infection; adjunctive TB vaccine; therapeutic TB vaccine; *mce1* operon; *mce1R*

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Introduction

The World Health Organization (WHO) estimated that in 2008, 8.9–9.9 million new cases of tuberculosis (TB) and 1.1–1.7 million deaths among HIV-negative people occurred [1]. These cases include people who progressed to active disease within months after a new exogenous infection with *Mycobacterium tuberculosis* (primary disease), those who developed the disease many years after an infection (reactivation TB), and those whose TB recurred after completing a treatment course (relapse TB). All of them went through a period of incubation or latent infection before developing active disease. Preventing these infected or inadequately treated individuals from developing active disease during this latent “window” period has the potential to make a huge impact on TB control worldwide; it will not only prevent active disease in the infected individuals but also future transmissions from these individuals. However, the current standard treatment of latent TB infection (LTBI) with isoniazid takes 6–9 months, and it is associated with poor compliance and potential side effects [2]. The treatment of relapse TB is complicated by the high frequency of multidrug resistance. A more efficient and safer way to prevent reactivation TB or relapse is urgently needed.

One such approach may be post-exposure vaccination. At this time, the only approved TB vaccine is bacillus Calmette-Guerin (BCG), which is recommended by the WHO to be given at birth to prevent new disease in children. It is not effective in preventing reactivation or relapse TB. Nearly all new vaccines under development or clinical trials are designed to prevent TB from a new infection [3]. In 1999, Lowrie et al reported that a vaccine based on *Mycobacterium leprae* Hsp60 DNA had a therapeutic effect on mice infected with *M. tuberculosis* [4]. Okada et al used *M. tuberculosis* Hsp60 DNA and IL12 delivered in hemagglutinating virus of Japan (HJV)-envelope and liposomes to show reduced pathology and prolonged survival of mice and cynomolgus monkeys challenged with *M. tuberculosis* prior to the vaccination [5]. Neither of these studies tested their vaccine in an animal model in which latent infection was established. Earlier this year, Aagaard et al showed that a combination of 3 secreted proteins—Ag85B, ESAT-6, and Rv2660c (collectively called H56 vaccine)—significantly reduced bacterial load compared to controls in a modified version of the Cornell mouse model [6]. However, H56 was not able to completely sterilize the infection in mouse lungs.

Unfortunately, there is no ideal animal model of LTBI or reactivation disease. One widely used model to mimic human LTBI is the Cornell mouse model [7–10]. Although this model is highly dependent on the parameters used to establish infection, it has the advantage of achieving very low or undetectable numbers of bacilli and maintaining those low levels for many weeks [11]. This undetectable phase of infection is operationally defined as the latent infection state. However, one drawback to this model is that true reactivation disease or relapse rarely occurs when wild type *M. tuberculosis* strains are used.

To overcome these limitations, we developed a modified version of the Cornell mouse model that takes advantage of a mutant strain of *M. tuberculosis* that mimics one stage of the natural infection with wild type *M. tuberculosis* [12]. *M. tuberculosis* contains a 13-gene operon called *mce1*, which is negatively regulated by *mce1R* when *M. tuberculosis* is intracellular [13, 14]. A phylogenomic analysis of the operon suggests the operon to encode a putative ATP-binding cassette (ABC) transporter, possibly involved in lipid importation. Wild type *M. tuberculosis* expresses the *mce1R* gene during the early phase of infection, but the gene is later repressed by as yet unknown external signals [13, 15]. This natural repression of *mce1R* leads to expression of the *mce1* operon genes, which is associated with bacterial proliferation and disease progression in mice [15]. We thus created a mutant of *M. tuberculosis* H37Rv disrupted in *mce1R*. This mutant ($\Delta mce1R$) constitutively expresses the

mce1 operon genes and causes accelerated immunopathologic response and death in mice [15]. In the Cornell mouse model, this mutant cannot be recovered from mouse lungs or spleen in the first 3 weeks after 8 weeks of treatment [12]. Thus, for 3 weeks, this mutant satisfies the operational definition of latent infection. However, after this period, an infected group of mice predictably develops disease, with a large number of bacteria recovered from both lungs and spleen [12]. Thus, we proposed that this mutant can be used to efficiently screen for vaccine or drug candidates to prevent reactivation disease in the Cornell model [12]. Here, we sought to identify a vaccine that would prevent $\Delta mce1$ R from replicating after treatment. This study was thus designed as a proof-of-the-concept study to demonstrate that an adjunctive vaccine—a vaccine used together with drugs—could prevent reactivation and relapse TB.

Methods

Cloning and expression of Mce1A

Mce1A was previously shown to mediate the uptake into nonphagocytic mammalian cells of *E. coli* and other cargo molecules attached to it [16-19]. The rationale for the selection of this protein as the first vaccine candidate was that its cell uptake may facilitate efficient antigen-presentation and recognition by sensitized T cells. The protein has a highly hydrophobic region at its N-terminus, which interfered with its purification. Hence, we selected a region spanning amino acid positions 51 to 454 for purification. A gene segment of *mce1A* encoding this region was amplified by PCR from *Mycobacterium tuberculosis* H37Rv and the PCR product was cloned into an expression vector pQE30 (Qiagen, CA, USA) after digestion with restriction enzymes *SphI* (New England BioLabs, MA, USA) and *HindIII* (New England BioLabs). The vector pQE-*mce1A* was transferred into *E. coli* M15[pREP4] designed to express a protein with a His tag at the N-terminus.

To confirm protein expression, we lysed the recombinant *E. coli* in 200 μ g/ml lysozyme and resolved the lysate by 10% SDS-polyacrilamide gel electrophoresis. The resolved proteins on the gel were transferred onto polyvinylidene difluoride (PVDF) membrane (Roche Applied Science) for Western blot assay, according to a standard protocol.

Purification of Mce1A protein

The recombinant *E. coli* was incubated in 100 ml of Luria Broth liquid medium with 100 μ g/ml ampicillin and 25 μ g/ml kanamycin at 37°C overnight. The bacterial culture was pelleted by centrifugation, which was then lysed in 6 ml of B-PER reagent (Thermo Fisher Scientific, Rockford, IL). We found that the insoluble fraction of the lysate contained more of the Mce1A protein, and hence it was resuspended in 5 ml of 8M urea solution in PBS. This solution was then eluted in Ni-NTA column. The elution fractions contained approximately 250 μ g/ml of proteins, >90% of which was Mce1A.

To assure protein purity used to vaccinate mice, we used the column-purified Mce1A extracted from SDS-PAGE gel. We selected elution samples with the highest concentration of Mce1A from the batch preparation and resolved them by SDS-PAGE. The SDS-PAGE gel was incubated for 5 min in a solution containing 1% sodium carbonate, 30 min in 200 mM imidazole with 0.1% SDS and 1 min in 100 mM zinc sulfate. The Mce1A protein band was then cut out from the gel and placed in SDS-PAGE buffer. The gel was homogenized and then incubated at room temperature (RT) overnight. The homogenate was then dialyzed against PBS buffer (pH 7.4) with phenylmethanesulfonyl fluoride (Sigma-Aldrich) at RT for 6 hours and at 4°C overnight. The concentration of the purified Mce1A protein was measured by DC protein assay (Bio-Rad).

Bacterial culture and mouse infection

The *mce1R* mutant strain (Δ mce1R) was constructed from *Mycobacterium tuberculosis* H37Rv by a two-step counter-selection strategy as described in detail previously [20-22]. Δ mce1R was aerosolized into 8-week-old female BALB/c mice (Charles River) by the Inhalation Exposure System (Glas-co, Terre Haute, IN), as described previously [15]. The aerosol nebulizer was set to administer approximately 150-200 bacilli per mouse lung.

Cornell mouse model and post-exposure immunization

Mice infected with Δ mce1R were maintained in a Biosafety Level-3 (BL-3) animal laboratory at the Northwest Animal Facility at University of California, Berkeley. After four weeks of infection, the mice were given isoniazid (INH, 100 μ g/ml, Sigma-Aldrich) and pyrazinamide (PZA, 15mg/ml, Sigma-Aldrich) ad lib in drinking water for eight weeks.

At day 0, all mice were infected by the aerosol route as described above. At day 1 and end of week 4, three mice for each time point were randomly selected from this whole group for lung and spleen histopathological examination and bacterial culture. At 12, 15, and 18 weeks after the initial infection, 5 mice per group were randomly selected to receive 50 μ g (in 200 μ l PBS) of the purified Mce1A or 200 μ l of PBS (pH7.4), injected into their peritoneal cavity.

Colony forming unit (CFU) assay

At day 1, 4 weeks, and 12 weeks after the aerosol infection, right lung from 3 mice were homogenized and plated onto Middlebrook 7H11 agar with OADC (oleic acid-albumin-dextrose-catalase supplement) and 100 μ g/ml cycloheximide (Sigma-Aldrich) for cfu enumeration. This was done to assure comparability of the infectious inoculum in the different mouse groups. At 4 weeks after the infection (peak of infection), homogenized lungs from 3 mice were diluted appropriately with PBS buffer with 0.05% Tween80 and plated. We compared cfu's recovered from lungs of 5 mice per each group given Mce1A or PBS at 21 and 32 week after the initial infection. The plates were examined 23 days after each plate inoculation.

Histopathological examination

At 4, 12, 21, and 32 weeks of infection, left lung from each mouse per group were fixed in 10% formalin in PBS buffer and embedded in paraffin. The sectioned samples were stained with hematoxyline and eosin (H&E) for histological examination, and Ziehl-Neelsen for acid-fast bacilli. These sections and stains were prepared by Histology Consultation Service (Everson, Washington). The sections were then reviewed by a pathologist specializing in mouse pathology, blinded to the source of the specimens.

Results

Purification of Mce1A

The recombinant *E. coli* containing the vector pQE30-*mce1A* expressed a 43kDa protein found mostly in the inclusion body fraction of the lysate (data not shown). The Western blot assay confirmed this protein to be Mce1A. Purified Mce1A was used to vaccinate mice, as described below.

Colony forming unit (cfu) assay results in the Cornell mouse model

The average number of Δ mce1R bacteria recovered from each mouse at day 1 of infection (indicating effective infectious inoculum) was 273 (Table 1). This inoculum size was previously shown to induce latent infection after an 8-week course of antibiotics [12]. After

4 weeks, the number of cfu's recovered from lung and spleen reached a peak at about 10^7 and 10^5 per organ, respectively (Table 1). At 12 weeks after infection (end of 8-week treatment), no bacteria were recovered from lungs or spleen from any of the mice, indicating either complete sterilization or establishment of latent infection with $\Delta mce1R$.

Mice were given 50 μ g of purified Mce1A or 200 μ l of PBS at 1 day, 3 weeks, and 6 weeks after cessation of the drugs. At 21 weeks after infection (or 3 weeks after the last dose of the vaccine or PBS), no bacteria were recovered from lungs or spleen of any of the vaccinated mice, whereas 3 of 5 mice given PBS had 5-1153 cfu's per lung ($p=0.083$, Fisher's exact test, 1-tailed); no organism was recovered from spleen of any of the 5 mice given PBS (Table 1). The mean number of cfu's recovered from all lungs of mice given PBS vs Mce1A was 230 and 0, respectively ($p=0.054$, Kruskal-Wallis test).

At 32 weeks (or 14 weeks after the last dose of the vaccine or PBS), no bacteria were recovered from lungs of any of the 5 vaccinated mice, whereas lungs from 4 of 5 mice given PBS had 25-2131 cfu's per lung ($p=0.024$, Fisher's exact test). At 32 weeks, one cfu was recovered from spleen of only one mouse, while spleen of 4 of 5 mice grew 33-1735 cfu per organ ($p=0.10$) (Table 1). The mean number of cfu recovered from all lungs and spleen from mice given PBS was 593 and 536, respectively, while that of vaccinated mice was 0 and 0.2, respectively ($p<0.05$ for both, Kruskal-Wallis test).

Gross examination of mouse lungs

Many small granulomatous lesions were observed in lungs removed from mice at the end of treatment (12 weeks of infection; Figure 1a). At 21 weeks after infection, large yellowish granulomatous lesions, mostly confined to the middle lobules of lungs of vaccinated mice were visible (Figure 1b). Lungs of PBS- injected mice showed yellowish granulomas spreading to both middle and lower lobules (Figure 1c). At 32 weeks of infection, granulomatous lesions in both groups were visible in all lobes, but the lungs of PBS-injected mice exhibited larger lesions with greater involvement of the lung parenchyma (Figure 1e). The lung size of both groups after infection was larger than that of an uninfected mouse (Figure 1f).

Histopathology of mouse lungs

Lung sections were examined by a mouse pathologist blinded to the source from the following groups of mice: after 4 weeks (maximum infection period, just before treatment), 12 weeks (end of treatment), 21 weeks, and 32 weeks of aerosol infection. Large, coalescing granulomas occupying more than 70% of the lung parenchyma were visible in a sagittal lung section of a mouse infected for 4 weeks (Figure 2a). After 8 weeks of treatment, the lung sections showed resolution of the large lesions, and much of the lung parenchyma showed intact alveolar air spaces with small pockets of inflammation (Figure 2b).

At 21 weeks of infection (9 weeks after cession of treatment; 3 weeks after the third dose of vaccine or PBS), the lung sections of the Mce1A-vaccinated mice showed several small lymphoid aggregates with adjacent granulomas characterized by foamy alveolar macrophages (Figure 3). In Figure 3a, one focal granuloma of foamy macrophages with multinucleated giant cells and cholesterol clefts was visible. There was mild to moderate perivascular lymphoid cuffing and the lesions affected less than 25% of the lung. The lymphocyte aggregates were much denser than those observed in lung sections of PBS-administered mice infected for 21 weeks (Figure 3b).

At 21 weeks, lung sections of mice administered PBS showed large multifocal, coalescing granulomas characterized by foamy alveolar macrophages with adjacent lymphoid infiltrates (Figure 3b). In Fig. 3-1b, the large granuloma shows a central area composed of

macrophages, surrounded by lymphocytes and another layer of macrophages at the periphery. The macrophages in each granuloma were more numerous than those in vaccinated mice. The lesions affected 25-50% of the lung.

At 32 weeks, (20 weeks after cessation of treatment; 14 weeks after the third dose of vaccine or PBS), lung sections of vaccinated mice showed several small dense granulomas comprised mostly of lymphocytes with a small number of macrophages, some containing light brown pigment. Much of the alveolar air spaces were intact. The lesions affected <25% of the lung.

At 32 weeks of infection in PBS-administered mice, the lung sections showed large granulomatous lesions occupying nearly 50% of the lung (Figure 3d). Fig. 3-1d shows focal coalescing granulomas adjacent to the airway, composed of foamy alveolar macrophages surrounded by a dense lymphoid infiltrate and scattered fibrosis (Figure 3-2d). The granulomas were very large and well demarcated. There were also mild neutrophilic infiltrates and necrosis within the granulomas. Within the adjacent airways, there were degenerate macrophages. Remnants of similar large granulomas were present in the less affected regions of the lung.

Ziehl-Neelsen stain of mouse lungs showed no acid fast bacilli at 21 or 32 weeks after infection in vaccinated or PBS-administered mice (data not shown).

Discussion

M. tuberculosis disrupted in the negative regulator *mce1R* establishes latent infection and predictably causes active disease following cessation of an 8-week course of antibiotics in the Cornell mouse model [12]. For a period of at least 3 weeks after the end of treatment, $\Delta mce1R$ cannot be recovered from lungs or spleen (latent phase). Subsequently, bacterial replication resumes and progresses, which results in overt disease after less than 20 weeks of infection [12]. These results were reproduced in this study in mice given only PBS. The number of bacteria recovered after 32 weeks in the unvaccinated mice was similar ($\sim 10^3$) to that in mice infected for 32 weeks with the *mce1R* mutant in our previous report [12], demonstrating the consistency of this new infection model. In the 8-week treatment Cornell mouse model that uses wild type *M. tuberculosis*, the recovery of bacilli from lungs or spleen is unpredictable and no bacilli may be cultured for many months, if at all. True reactivation disease rarely occurs. Hence, with wild type *M. tuberculosis*, the Cornell model cannot be efficiently used to assess therapeutic or preventive modalities.

We took advantage of $\Delta mce1R$ in this model to identify a vaccine that could suppress the replication of $\Delta mce1R$ and prevent disease. The relevance of this mutant to wild type infection is that, in vivo, wild type *M. tuberculosis* undergoes a stage of infection that is exhibited by $\Delta mce1R$. That is, about 8 weeks into a mouse infection, the *mce1R* gene of wild type *M. tuberculosis* is repressed (thus functionally equivalent to $\Delta mce1R$) and the *mce1* operon genes are turned on; the expression of the operon genes is consistently associated with bacterial proliferation and disease progression [15].

We found that Mce1A, one of the cell wall proteins encoded by a gene in the *mce1* operon, effectively prevented post-treatment relapse and suppressed bacterial replication. Since $\Delta mce1R$ constitutively expresses Mce1A, the protective effect of Mce1A is not surprising. In fact, this Mce1A may also protect against wild type *M. tuberculosis* because, as mentioned above, the wild type *M. tuberculosis mce1A* gene is expressed about 8 weeks into infection in mice [15]. Thus, if during wild type *M. tuberculosis* infection, the mouse is already sensitized against Mce1A, a wild type strain that expresses Mce1A could be immunologically recognized and hence prevented from proliferating.

We selected Mce1A as a vaccine candidate because, with its cell penetrating peptide motif, it is readily taken up by mammalian cells [16-19]. Such a protein may be efficiently processed intracellularly and presented as antigens that may induce a strong protective T cell response. If T cells are primed with Mce1A before bacterial proliferation, they could help macrophages eliminate the bacilli that express Mce1A.

Here, we delivered the vaccine intraperitoneally (IP). Of course, this would not be an efficient way to deliver a vaccine in humans. We note that this study was not undertaken to mimic how this protein would ultimately be applied in human trials. We had actually delivered the vaccine to mice subcutaneously in an earlier experiment (data not shown). The Mce1A protein was able to achieve sterilizing protection in lungs but the number of cfu counts from the spleen exceeded 100 per spleen at 32 weeks of infection (data not shown). In this study, we found only 1 cfu from spleen of one vaccinated mouse.

In this study, the most striking effect of the vaccine was the bacterial sterilization in the lungs. No bacteria were recovered from lungs of 10 vaccinated mice over a 32-week period (Table 1). In fact, only 1 cfu was recovered from spleen of one mouse among 5 vaccinated mice at 32 weeks. To date, no vaccine candidates have shown a sterilizing effect in mouse lungs against *M. tuberculosis* challenge. In the most recent report of a vaccine (H56) used to prevent reactivation TB in a mouse model, the most optimal results reported mean cfu of more than 10 per lung (0 to >100) in the vaccinated group of 12 mice [6].

The most dramatic difference in lung histopathology we observed was in the cell population that comprised the granulomas (Fig. 3). At the end of 8 weeks of treatment, the lungs showed small areas of inflammation comprising less than 10% of the lung parenchyma (Fig. 2b). At 21 and 32 weeks of infection, the inflammation and granulomas in unvaccinated mice progressively increased in size, which suggests recruitment of both macrophages and lymphocytes after the treatment period. On the other hand, in the vaccinated mice, the lesions actually became smaller after the end of treatment, with a very small number of macrophages, suggesting no further recruitment. The presence of a large number of macrophages appears to correlate with lack of protection.

With relevance to human patients, the mouse model that we used here is more akin to post-treatment relapse disease than reactivation TB from LTBI. That is, the mice were allowed to develop disease before treatment, and developed disease again after cessation of the drugs. Relapse generally occurs in about 8% of treated patients in most TB endemic countries, and in about 13% if the prevalence of multidrug-resistant (MDR) TB in the target population is more than 3% [23]. This number increases in those who do not complete a full treatment course, who are HIV infected, who have MDRTB, or if the treatment regimen does not include both isoniazid and rifampicin [23-26].

The human host is relatively efficient in controlling *M. tuberculosis* infection after exposure. Thus, if a vaccine can augment host immunity and clear the residual bacterial load after treatment, such an approach could potentially be used as an adjunctive therapy to greatly reduce the probability of reactivation in those with LTBI or relapse TB in those who completed treatment. Such a vaccine may also be used to shorten the duration of treatment of active disease or LTBI from the standard 6-9 months. Furthermore, since a large proportion of people who develop multidrug resistant TB has a history of previous TB treatment, a post-treatment vaccine could potentially prevent and control drug-resistant TB. The $\Delta mce1R$ mutant we constructed provides an opportunity to examine these new approaches using the mouse model to develop protocols to more effectively treat LTBI and prevent relapse TB.

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References

1. World Health Organization. Global tuberculosis control: a short update to the 2009 report. 2009. cited; Available from: http://www.who.int/tb/publications/global_report/2009/update/en/index.html
2. Lobue P, Menzies D. Treatment of latent tuberculosis infection: An update. *Respirology*. 2010; 15:603–22. [PubMed: 20409026]
3. Kaufmann SH, Hussey G, Lambert PH. New vaccines for tuberculosis. *Lancet*. 2010; 375:2110–9. [PubMed: 20488515]
4. Lowrie DB, Tascon RE, Bonato VL, et al. Therapy of tuberculosis in mice by DNA vaccination. *Nature*. 1999; 400:269–71. [PubMed: 10421369]
5. Okada M, Kita Y, Nakajima T, et al. Novel prophylactic and therapeutic vaccine against tuberculosis. *Vaccine*. 2009; 27:3267–70. [PubMed: 19200841]
6. Aagaard C, Hoang T, Dietrich J, et al. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. *Nat Med*. 2011; 17:189–94. [PubMed: 21258338]
7. McCune R, Tompsett R, McDermott W. Fate of *Mycobacterium tuberculosis* in mouse tissues as determined by the microbial enumeration technique. II The conversion of tuberculous infection to the latent state by the administration of pyrazinamide and a companion drug. *J Exp Med*. 1957; 104:763–802. [PubMed: 13367342]
8. McCune RM, Tompsett R. Fate of *Mycobacterium tuberculosis* in mouse tissues as determined by the microbial enumeration technique. I. The persistence of drug-susceptible tubercle bacilli despite prolonged antimicrobial therapy. *J Exp Med*. 1957; 104:737–62. [PubMed: 13367341]
9. McCune RM, Feldmann FM, Lambert HP, McDermott W. Microbial persistence. I. The capacity of tubercle bacilli to survive sterilization in mouse tissues. *J Exp Med*. 1966; 123:445–68. [PubMed: 4957010]
10. McCune RM, Feldmann FM, McDermott W. Microbial persistence. II. Characteristics of the sterile state of tubercle bacilli. *J Exp Med*. 1966; 123:469–86. [PubMed: 4957011]
11. Scanga CA, Mohan VP, Joseph H, et al. Reactivation of latent tuberculosis: variations on the Cornell murine model. *Infect Immun*. 1999; 67:4531–8. [PubMed: 10456896]
12. Cheigh CI, Senaratne R, Uchida Y, et al. Posttreatment reactivation of tuberculosis in mice caused by *Mycobacterium tuberculosis* disrupted in *mce1R*. *J Infect Dis*. 2010; 202:752–9. [PubMed: 20629532]
13. Casali N, White AM, Riley LW. Regulation of the *Mycobacterium tuberculosis* *mce1* operon. *J Bacteriology*. 2006; 188:441–9.
14. Casali N, Riley LW. A phylogenomic analysis of the Actinomycetales *mce* operons. *BMC Genomics*. 2007; 8:60. [PubMed: 17324287]
15. Uchida Y, Casali N, White A, et al. Accelerated immunopathological response of mice infected with *Mycobacterium tuberculosis* disrupted in the *mce1* operon negative transcriptional regulator. *Cell Microbiol*. 2007; 9:1275–83. [PubMed: 17223927]
16. Arruda S, Bomfim G, Knights R, Huima-Byron T, Riley LW. Cloning of an *M. tuberculosis* DNA fragment associated with entry and survival inside cells. *Science*. 1993; 261:1454–7. [PubMed: 8367727]
17. Chitale S, Ehrst S, Kawamura I, Fujimura T, Shimono N, Anand N, Lu S, Cohen-Gould L, Riley LW. Recombinant *Mycobacterium tuberculosis* protein associated with mammalian cell entry. *Cellular Microbiology*. 2001; 4:247–54. [PubMed: 11298648]
18. Casali N, Konieczny M, Schmidt MA, Riley LW. Invasion activity of a *Mycobacterium tuberculosis* peptide presented by the *Escherichia coli* AIDA autotransporter. *Infect Immun*. 2002; 70:6846–52. [PubMed: 12438361]

19. Lu S, Tager LA, Chitale S, Riley LW. A cell penetrating peptide derived from mammalian cell entry protein of *Mycobacterium tuberculosis*. *Anal Biochem*. 2006; 353:7–14. [PubMed: 16620748]
20. Shimono N, Morici L, Casali N, Cantrell S, Sidders B, Ehrt S, Riley LW. Hypervirulent mutant of *Mycobacterium tuberculosis* resulting from disruption of the *mce1* operon. *Proc Natl Acad Sci USA*. 2003; 100:15918–23. [PubMed: 14663145]
21. Casali N, White AM, Riley LW. Regulation of the *Mycobacterium tuberculosis* *mce1* operon. *J Bacteriol*. 2006; 188:441–9. [PubMed: 16385033]
22. Parish T, Stoker NG. Use of a flexible cassette method to generate a double unmarked *Mycobacterium tuberculosis* *tlyA plcABC* mutant by gene replacement. *Microbiology*. 2000; 146:1969–75. [PubMed: 10931901]
23. Mak A, Thomas A, Del Granado M, et al. Influence of multidrug resistance on tuberculosis treatment outcomes with standardized regimens. *Am J Respir Crit Care Med*. 2008; 178:306–12. [PubMed: 18511706]
24. Kim DH, Kim HJ, Park SK, et al. Treatment outcomes and long-term survival in patients with extensively drug-resistant tuberculosis. *Am J Respir Crit Care Med*. 2008; 178:1075–82. [PubMed: 18703792]
25. Charalambous S, Grant AD, Moloi V, et al. Contribution of reinfection to recurrent tuberculosis in South African gold miners. *Int J Tuberc Lung Dis*. 2008; 12:942–8. [PubMed: 18647455]
26. Panjabi R, Comstock GW, Golub JE. Recurrent tuberculosis and its risk factors: adequately treated patients are still at high risk. *Int J Tuberc Lung Dis*. 2007; 11:828–37. [PubMed: 17705947]

Highlights

An *M. tuberculosis* mutant was efficiently used to test a TB vaccine candidate; a combination of a vaccine and drugs prevents reactivation and relapse TB in mice; this combination achieved a sterilizing effect in mouse lungs; an adjunctive vaccine could greatly shorten active and latent TB treatment.

Figure 1. Gross pathology of mouse lungs. (a) Lung of a mouse infected for 12 weeks, (end of an 8-week treatment course). (b) Lung of mouse infected for 21 weeks, vaccinated with Mce1A, or (c) administered PBS. (d) Lung of mouse infected for 32 weeks, vaccinated with Mce1A, or (e) given PBS. (f) Lung from an uninfected mouse.

Figure 2. Histology (H&E stain) of sagittal lung sections of mice infected for 4 weeks, just before the initiation of treatment (a), and for 12 weeks, at the end of an 8-week treatment course (b).

Figure 3. Histology of mouse lung sections (H&E stain). Panels a and b show lung sections of mice infected for 21 weeks; lung section of mouse vaccinated with Mce1A at 12.5 × (a1) and 200× (a2) magnification; lung section of mouse given PBS at 12.5× (b1) and 200× (b2) magnification. Panels c and d show lung sections of mice infected for 32 weeks; lung section of mouse vaccinated with Mce1A at 12.5 × (c1) and 200× (c2) magnification; lung section of mouse given PBS at 12.5× (d1) and 200× (d2) magnification. Arrow in 12× panels indicates the site of magnification shown in 200× panels.

Figure 4.

Scatter plot of colony forming units (cfu) recovered from lungs (A) and spleen (B) of mice infected with $\Delta mce1R$ and given *mce1A* or PBS at 12, 15, and 18 weeks of infection. All mice were infected by the aerosol route and a group of mice were randomly selected for organ histopathologic examination and cfu enumeration at the indicated time points. At weeks 21 and 32, five mice per group were examined, while at day 1 and weeks 4 and 12, three mice per group were examined. The absence of a dot indicates 0 cfu recovery from the animal at the indicated time points.