Review

*Mycobacterium tuberculosis* proteins involved in cell wall lipid biosynthesis improve BCG vaccine efficacy in a murine TB model

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**ABSTRACT**

Objectives: Advances in tuberculosis (TB) vaccine development are urgently required to enhance global disease management. We evaluated the potential of *Mycobacterium tuberculosis* (*M. tb*)-derived protein antigens Rv0447c, Rv2957 and Rv2958c to boost BCG vaccine efficacy in the presence or absence of glucopyranosyl lipid adjuvant formulated in a stable emulsion (GLA-SE) adjuvant.

Methods: Mice received the BCG vaccine, followed by Rv0447c, Rv2957 and Rv2958c protein boosting with or without GLA-SE adjuvant 3 and 6 weeks later. Immune responses were examined at given time points. 9 weeks post vaccination, mice were aerosol-challenged with *M. tb*, and sacrificed at 6 and 12 weeks to assess bacterial burden.

Results: Vaccination of mice with BCG and *M. tb* proteins in the presence of GLA-SE adjuvant triggered strong IFN-γ and IL-2 production by splenocytes; more TNF-α was produced without GLA-SE addition. Antibody responses to all three antigens did not differ, with or without GLA-SE adjuvant. Protein boosting without GLA-SE adjuvant resulted in vaccinated animals having better control of pulmonary *M. tb* load at 6 and 12 weeks post aerosol infection, while animals receiving the protein boost with GLA-SE adjuvant exhibited more bacteria in the lungs.

Conclusions: Our data provides evidence for developing Rv2958c, Rv2957 and Rv0447c in a heterologous prime-boost vaccination strategy with BCG.

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Introduction

Tuberculosis (TB) vaccine development is a highly challenging task, and has met with setbacks in recent years. Clinical trials evaluating novel vaccine candidates have, however, taught the global TB community lessons that collectively improve our knowledge of protective, anti-TB immune responses in humans. The most significant observation in a majority of vaccine efficacy studies is that the existing TB vaccine, Bacille Calmette-Guérin (BCG) appears to be at least as effective as novel candidates that have undergone phase II clinical testing in infants.1,2 Nevertheless, BCG vaccination appears to be variably protective against TB meningitis in children (at least 70% protection against active TB disease)3,4 but not against pulmonary TB in adults.5 Thus, genetically modifying or boosting (viral-vectorized, purified protein antigens) the potency of the BCG vaccine to induce durable memory responses in individuals is becoming a highly promising strategy.6–8 In addition, newer strategies aiming at generating attenuated strains of Mycobacterium tuberculosis as vaccine candidates have also witnessed initial success.9

Host- and pathogen-related factors appear to affect the longevity of protective immune responses conferred by vaccination against TB. The quality of the cellular immune response (orchestrated by T cells) plays a critical role in barraging the spread of M. tb infection from the lungs.10,11 In addition, enhanced antibody responses directed against M. tb antigens are equally important for controlling disseminated disease as well as intracellular killing.12 Importantly, the nature or localisation of M. tb antigens themselves (intracellular vs. extracellular vs. secreted) influence the establishment of effective cellular and humoral immune responses.13,14 Another highly influential component in optimising vaccination strategies is the use of adjuvants. Glucopyranosyl Lipid Adjuvant-stable emulsion (GLA-SE) is a synthetic toll-like receptor 4 agonist formulated in a stable, squalene oil-in-water emulsion.15 The ability of GLA-SE to drive Th1 immune responses is well-documented,16,17 while its potential in vaccinology has been evaluated in studies using preclinical animal models of influenza,18 human immunodeficiency virus19 and TB20 infection.

We have previously reported a peptide microarray-based screen to map humoral immune responses from healthy individuals as well as those with active TB directed against M. tb targets.14 In this study, CFA-synthase (UfA11; Rv0447c) and two glycosyltransferases (Rv2957 and Rv2958c), which are involved in the synthesis, transport and maintenance of glycolipids within the mycobacterial cell wall,21–23 were also identified as novel M. tb antigens with immunological and clinical relevance, based on strong recognition by serum IgG antibodies from patients with TB (for convenience, we have termed the antigens as the ‘KI-TB antigens’). This finding spurred a series of in vitro immunological assessment (i.e. cytokine production by T cells in whole blood to antigenic stimulation, human leukocyte antigen (HLA)-DR binding assays of specific epitopes in humans) with Rv0447c, Rv2957 and Rv2958c using peripheral blood from patients with TB, persons with latent TB infection (LTBI) and healthy individuals.24–26 The results from these studies established that Rv0447c, Rv2957 and Rv2958c participate in orchestrating clinically relevant immune responses in human TB by potentiating CD4+ and CD8+ T-cell responses, with likely implications in protection against clinical disease. Importantly, the BCG vaccine also expresses all three proteins, although the Rv2958c gene is aberrantly transcribed due to a frameshift mutation.27,28 In this study, we report the testing of Rv0447c, Rv2957 and Rv2958c as potential novel vaccine candidates in a preclinical mouse model of TB in terms of immunogenicity and protective efficacy.

Materials and Methods

Animals and ethical clearance

Female, wild type C56BL/6 mice (bred in-house at Karolinska University Hospital Huddinge, Stockholm) were housed in individually ventilated cages during the course of the study. Animals were provided with a constant supply of clean drinking water and fresh chow. All experimental procedures involving mice were performed in adherence to requirements of the Swedish Department of Agriculture (jordbruksverket; approved ethical permit number N273/13). Mice were sacrificed by isoflurane anaesthesia and cervical dislocation. All efforts were made to ensure minimized suffering and pain.

Reagents and culture media

Middlebrook 7H9 liquid medium supplemented with 10% OADC, 2% glycerol and 0.05% Tween 80 (7H9 complete medium (7H9C)) as well as Middlebrook 7H11 solid medium supplemented with 10% OADC and 2% glycerol (7H11C) were purchased from the Karolinska University Hospital substrate unit. RPMI Glutamax medium, fetal bovine serum (FBS) and antibiotics for tissue culture (penicillin/streptomycin) were purchased from Life Technologies. Full-length recombinant M. tb proteins (Rv0447c, Rv2957 and Rv2958c) were purchased from GenScript (Hong Kong). GLA-SE adjuvant was purchased from the Infectious Disease Research Institute, IDRI (Seattle, WA, USA).

Vaccinations

AERAS TB Vaccines Foundation (Rockville, MD, USA) kindly provided seed stocks of the Mycobacterium bovis Bacille Calmette-Guerin (BCG) Danish strain 1331. BCG was cultured in 7H9C medium and grown to mid to late log-phase (OD600 0.6–0.8). Mycobacterial cultures were then washed with PBS buffer containing 0.05% Tween 80 (PBST80) and resuspended in 7H9C medium supplemented with 16% glycerol and stored at −80 °C. For vaccinations, 6–7 week old mice were given a subcutaneous injection of either 1×10⁶ colony forming units (CFU) of the BCG vaccine or phosphate-buffered saline (PBS; sham) in the scruff of the neck. 3 weeks later, the animals were subcutaneously immunised with a whole-protein antigen mix containing 2 μg of each M. tb protein (R2958c + Rv2957 + Rv0447c) with or without 5 μg GLA-SE adjuvant, a highly potent TLR4 agonist.29 A second round of protein administration (booster) was given 3 weeks after the initial injection (with/without GLA-SE). A schematic representation of the vaccination schedule is shown below:
**Splenocyte culture and analysis of T cells**

The animals were sacrificed nine weeks after BCG priming and two rounds of protein immunisation. Spleens were aseptically removed, and put in separate 15 ml Falcon tubes containing RPMI 1640 medium (Life Technologies) supplemented with 10% FCS, penicillin and streptomycin (R10+). Each spleen was washed using a sterile 70 µm cell strainer (BD) and 2 ml syringe plunger in a petri dish to prepare single-cell suspensions. The cell strainer was then washed with R10+, the flow through transferred to the respective 15 ml Falcon tube and centrifuged at 1300 rpm for 5 minutes at 4°C. ACK lysis buffer (Life Technologies) was added to the cells, and incubated at room temperature (RT) for 1–2 minutes. The tubes were then resuspended with R10+ medium to stop the reaction, followed by centrifugation at 1300 rpm for 5 minutes at RT. After resuspending and counting the cells, 2 × 10^5 splenocytes were seeded in a 96-well round-bottom plate, and re-stimulated in vitro with 1 µg/ml of the antigen mix (R2958c + Rv0447c) for 72 hours at 37°C with 5% CO₂. At the end of the incubation period, the plate was centrifuged at 1600 rpm and the resulting supernatant transferred to a non-sterile 96-well round-bottom plate for storage at −20°C. The remaining cell pellets were resuspended in FACS buffer (2% FCS in PBS) and centrifuged at 1300 rpm for 5 minutes. This was followed by staining of the cells with the following antibodies (each diluted at 1:200): CD4 PE-CF594 (BD Biosciences), CD3 PerCP-Cy5.5 (BioLegend) and CD8 PE-Cy7 (BioLegend) on ice for 15–20 minutes. After washing the stained cells, BD Cytofix/Cytoperm solution was added to them, and left to incubate on ice for 20 minutes, followed by one wash with 1 x perm wash (BD Biosciences). The cells were then resuspended in 1 x perm wash containing 1:200 diluted antibodies for intracellular staining (TNFα-FITC, IFNγ-BV421, IL-2-APC; all purchased from BioLegend) and incubated on ice for 30 minutes. After one wash with 1 x perm wash, the stained cells were resuspended in FACS buffer, and filtered through a 35 µm cell strainer capped onto a 12 × 75 mm tube (BD Biosciences). Cells were acquired on a BD FACS Aria flow cytometer (BD Biosciences) followed by analysis using FlowJo software (Treestar Inc.).

**Cytokine analysis**

ELISA kits were purchased from Mabtech (IFNγ and IL-2) and E-Biosciences (TNFα), and assays for measuring cytokine content in splenocyte cell culture supernatants were performed according to the manufacturer's instructions.

**Blood serum preparation**

Whole blood from sacrificed animals was collected in a microcentrifuge tube, and left to clot at RT for 30 minutes. The tubes were then centrifuged at 2000 rpm for 10 minutes in a refrigerated (4°C) microcentrifuge, and the resulting serum layer was immediately transferred to a clean capped polypropylene tube for storage at −80°C.

**Detection of antigen-specific IgG in mouse serum by ELISA**

Nunc MaxiSorp ELISA plates (ThermoFisher) were coated with 1 µg/ml stock solution of M. tb antigens (Rv0447c, Rv2957c or Rv2958c) prepared in PBS and incubated for 1 hour at 37°C. The assay plate was washed 3 times with PBS-0.05% Tween 20 (PBST20) buffer, followed by blocking with the same buffer and incubation at RT for 1 hour. After thorough washing with PBST20 buffer, 1:100-diluted murine sera (diluted in dilution buffer (PBS 0.1%-BSA-0.05% Tween 20)) was added to each well on the assay plate. An 8-point serial dilution of IgG standards at a ratio of 1:2 (1000 to 8 ng/ml) was prepared from a 1000 ng/ml stock solution of recombinant murine IgG (Mabtech) in PBS, and each dilution was added in duplicates to the respective wells on the assay plate. The assay plate was then incubated for 2 hours at RT, and washed thoroughly with PBST20 buffer. For detection, alkaline phosphate-conjugated anti-human IgG monoclonal antibody (Mabtech) was diluted at a ratio of 1:1000 in dilution buffer and added to all wells, followed by 1-hour incubation at RT. The assay plate was then washed 5 times with PBST20 buffer. Substrate solution (p-nitrophenyl phosphate tablets (PNPP; ThermoFisher) dissolved in 1 x diethanolamime substrate buffer (ThermoFisher) was added to the assay plate and incubated for 15 minutes at RT. The enzymatic reaction was stopped with 1N NaOH, and the optical density measured at 405 nm.

**Aerosol infection of mice with virulent M. tb**

Nine weeks after BCG/sham-vaccination and 2 rounds of protein immunisation, the animals were aerosol-challenged with 100-200 CFU of virulent M. tb H37Rv using a nose-only exposure system, as previously published.28,29 At 6 and 12 weeks post aerosol challenge, the animals were sacrificed, and lungs and spleens were aseptically removed. The organs were separately homogenised using a sterile 70 µm cell strainer (BD) and 2 ml syringe plunger in a petri dish containing PBS +0.05% Tween 80 buffer. Serial dilutions of the homogenates were prepared in the same buffer for plating onto Middlebrook 7H11 medium supplemented with 10% OADC (BD), 10 µg/ml ampicillin and 10 µg/ml cycloheximide. Plates were incubated at 37°C for 3-4 weeks prior to enumerating CFU. A schematic representation of the protection efficacy study is presented below:
Statistical analysis

Data was graphed and analysed using the Graph Pad Prism 6 software. A p value of 0.05 or lower was considered significant.

Results

We noticed in immunogenicity studies that BCG priming alone was not able to induce production of IFN-γ and IL-2 by splenocytes following a 3-day exposure to Rv0447c, Rv2957 and Rv2958c in vitro (Figure 1). However, with two administrations of the protein antigens (with or without GLA-SE adjuvant), the production of both cytokines was markedly increased (Figure 1). Interestingly, only splenocytes from animals that received BCG alone, and those that received the antigens alone without GLA-SE adjuvant mounted a strong TNF-α response. Also, animals vaccinated with BCG and boosted with Rv0447c, Rv2957 and Rv2958c in

Figure 1. Immunological assessment of cytokine production by splenocytes in culture supernatants. Mice were primed with BCG and given two boosts with KI-TB antigens 3 and 6 weeks later, with or without GLA-SE adjuvant. 3 weeks after the second boost (9 weeks post BCG priming), spleens of vaccinated mice were aseptically removed, and splenocytes were isolated. Cells were incubated with 1 μg/ml KI-TB antigen mix for a 72-hour period, after which the culture supernatants were collected for cytokine analysis by ELISA. IFN-γ and IL-2 production, respectively are significantly higher in the group that received BCG priming and the antigen boost with GLA-SE adjuvant as well as the group that received only the antigen boost with GLA-SE, as compared to the group which received only BCG (for priming). TNF-α production, on the other hand, is significantly higher in the group that received only the antigen boost without the GLA-SE antigen, as compared to the group that received BCG priming, followed by the antigen boost with GLA-SE adjuvant. Shown are the means and standard error of mean (SEM) of four mice per group (Kruskal-Wallis test of means; *p < 0.05; **p < 0.01).
the absence of GLA-SE adjuvant exhibited a stronger TNF-α response to in vitro antigen re-stimulation compared to animals receiving the same vaccination regimen with GLA-SE adjuvant (Figure 1). Similarly, splenocytes from animals vaccinated with the antigens without GLA-SE adjuvant produced more TNF-α compared to those receiving antigens with GLA-SE adjuvant. An important observation at this stage of the study was that inclusion of GLA-SE adjuvant in the protein-boosting step triggered a preferential IFN-γ and IL-2 response by splenocytes of vaccinated animals.

Next, we wanted to determine the T cell subset responsible for cytokine production driven by booster vaccination with Rv0447c, Rv2957 and Rv2958c in the presence or absence of GLA-SE adjuvant and BCG priming. In this regard, we observed both frequencies of cytokine-producing T cells as well as the absolute cell counts. Boosting BCG vaccination with a combination of Rv0447c, Rv2957 and Rv2958c with GLA-SE adjuvant led to a general increase in functional (IFN-γ+) antigen-specific CD8+ T cell responses (Figure 2). In the absence of GLA-SE adjuvant, CD4+ T cell responses appeared to have further increased, in particular T cells producing IL-2 and TNF-α (Figure 2). IL-2-producing CD8+ T cell responses also increased after boosting with Rv0447c, Rv2957 and Rv2958c but in the absence of GLA-SE adjuvant.

Immunisation with the protein antigens alone (without priming with BCG) emulated anti-Rv2957 serum IgG titres comparable to the BCG-primed group, in the presence or absence of GLA-SE adjuvant (Figure 3). For the recognition of Rv2958c by serum IgG, priming the animals with BCG and boosting with the protein antigens and GLA-SE adjuvant was superior to a heterologous prime-boost without GLA-SE adjuvant. We also observed that the protein antigens alone are able to induce a strong serum IgG response to Rv0447c when administered with GLA-SE adjuvant (Figure 3). These results prompted us to conclude that Rv0447c, Rv2957 and Rv2958c alone proved to be very immunogenic, as well as their induction of IFN-γ and IL-2, which is particularly interesting for TB.

In the absence of GLA-SE adjuvant, vaccination with BCG + KI antigens performed better than BCG or KI antigens alone, based on lung CFU results obtained 6 weeks post challenge. We however observed no differences in mycobacterial load in the spleen (Figure 4). At 12 weeks post M. tb challenge, vaccination with BCG + KI-TB antigens continued to do better than BCG or KI-TB antigens alone in reducing bacterial burden in the lungs, while in the spleen, it was effective as BCG or KI-TB antigens alone. Animals that were vaccinated with BCG + KI-TB antigens in the presence of GLA-SE adjuvant had sustained control of bacterial burden in the lungs and spleen between 6 and 12 weeks post M. tb challenge in comparison

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**Figure 2. Analysis of CD4+ and CD8+ T-cell responses induced by BCG priming and KI-TB antigen boosting.** Mice were primed with BCG and given two boosts with KI-TB antigens 3 and 6 weeks later, with or without GLA-SE adjuvant. 3 weeks after the second boost (9 weeks post BCG priming), spleens of vaccinated mice were aseptically removed, and splenocytes were isolated. Cells were incubated with 1 ug/ml KI-TB antigen mix (1 μg/ml per antigen; total of 3 μg/ml antigen mix) for a 72-hour period, after which the cell pellets were collected for intracellular cytokine staining analysis (IFN-γ, IL-2, TNF-α+ T cells) by flow cytometry. Cell frequencies (%) and absolute cell counts were analysed. Shown are the means and standard error of mean (SEM) of four mice per group (Kruskal-Wallis test of means; *p < 0.05; **p < 0.01).
with animals receiving the BCG alone (Figure 5). Furthermore, vaccination with BCG + KI-TB antigens in the presence of GLA-SE appeared to achieve significantly better control of bacterial burden in the spleen at 12 weeks post M. tb challenge.

Discussion

Vaccination strategies focused on improving the BCG vaccine form an integral component of the mainstay approaches to tackle TB. Administering BCG as a priming agent can generate a large repertoire of antigen-specific memory immune cells capable of producing Th1 cytokines i.e. IFN-γ, IL-2 and TNF-α or IgG antibodies, respectively.\(^{30,31}\) In order to sustain this response in the host, boosting with recombinant viral constructs expressing M. tb antigens,\(^{17}\) or simply administering a booster shot with purified recombinant M. tb proteins\(^{32}\) has been promising at early clinical evaluation stages. There is only one BCG replacement vaccine candidate in clinical trials, VPM1002, which incorporates superior immunogenicity and safety in individuals,\(^{33}\) while a new study is underway to test its efficacy in HIV-exposed infants (Clinicaltrials.gov identifier: NCT02391415).

In the present report, we show that boosting the BCG vaccine with recombinant M. tb proteins involved in mycobacterial cell wall synthesis and maintenance, namely Rv0447c, Rv2957 and Rv2958c leads to better control of M. tb burden in the lungs. Although use of the toll-like receptor 4 agonist GLA-SE as adjuvant strongly promoted antigen-specific IFN-γ production by T cells, it did not appear to enhance the anti-TB effect of the M. tb proteins — based on pulmonary M. tb load reduction inferior to that of animals vaccinated with BCG alone. Interestingly, TNF-α production by CD4 and CD8 T cells, which was more pronounced in animals receiving the BCG vaccine and protein boost without GLA-SE adjuvant, suggested a better control of pulmonary bacterial load compared to animals vaccinated with BCG and boosted with the protein antigens in the presence of GLA-SE adjuvant. Initial control of M. tb infection certainly seems to require TNF-α production, as seen in reactivation TB in individuals receiving anti-TNF-α antibody therapy for rheumatoid arthritis.\(^{34,35}\) Considering that serum IgG responses to Rv0447c, Rv2957 and Rv2958c were not different between animals receiving the BCG vaccine and protein boost with or without GLA-SE, we suggest that humoral immune responses to these antigens may not have a role to play in protecting against pulmonary TB. The M. tb proteins in question are intracellular and may be processed and presented via the MHC-I/II pathways, concomitantly supporting the strong T-cell responses observed in this study.

In general, the increase in cytokine producing antigen-specific T-cell frequencies correlated well with the absolute cell counts; this may reflect a true increase in the number of functional immune cells induced by the vaccination approach. Our results resonate with previous findings that high IFN-γ production by CD4 T cells after vaccination does not necessarily

![Figure 3. Detection of antigen-specific IgG in sera of vaccinated mice. Mice were primed with BCG and given two boosts with KI-TB antigens 3 and 6 weeks later, with or without GLA-SE adjuvant. 3 weeks after the second boost (9 weeks post BCG priming), whole blood was collected and serum was prepared for detecting antigen-specific IgG against the KI-TB antigens using an in-house ELISA method. Shown are the means and standard error of mean (SEM) of 3-4 mice per group.](image-url)
translate to superior anti-TB vaccine efficacy in the murine TB model. Another interesting observation was the IL-2 production by T cells from vaccinated mice. We noticed more IL-2 was released into the splenocyte culture medium than retained in the cells with GLA-SE adjuvant. However, without GLA-SE adjuvant, more IL-2 appeared to be retained within the T cells based on intracellular cytokine staining and ELISA, indicating that without GLA-SE, IL-2 production may be slightly delayed. This may not affect the protective efficacy of BCG vaccination boosted with Rv2958c, Rv2957 and Rv0447c without the GLA-SE adjuvant, since better reduction of mycobacterial load in the lungs was observed in the mice vaccinated with the KI-TB antigens without the GLA-SE adjuvant. Similarly, the high amount of IFN-γ detected in the splenocyte culture supernatant after 3 days of culture with the antigen mix and GLA-SE adjuvant indicates that the adjuvant might accelerate IFN-γ production, suggesting a rapid increase in systemic cytokine levels in the vaccinated host.

We also did not observe superior advantage of GLA-SE adjuvant in enhancing antibody responses to any of three antigens tested. Previous work performed in our laboratory reported induction of serum IgG antibodies from patients with TB recognising single epitopes of Rv2957 on a peptide microarray platform. It is possible that this antigen is differentially processed in humans as opposed to mice, in addition to the fact that active infection with M. tb in humans may have a different profile of antigen processing and presentation compared to the 'experimental vaccination' in mice. Thus, the linear epitopes of M. tb antigens recognised by IgG molecules in sera of patients with TB is likely to differ to those occurring in mice used in these preclinical studies.

The amount of antigen-specific IgG in sera of mice vaccinated with BCG + antigens (with/without GLA-SE) was not much higher than antigen-specific IgG induced by BCG alone. In fact, the actual concentration of IgG antibodies recognising Rv2957 (as well as Rv2958c and Rv0447c) was induced by BCG vaccination, with and without GLA-SE adjuvant (as compared to our studies using PBS as the baseline control, data not shown). The presence of circulating antigen-specific IgG indicates that Rv2958c, Rv2957 and Rv0447c can on their own (without adjuvant) induce measurable humoral immune responses – which is a key desirable feature of novel TB vaccines.

The results presented here further strengthen and are in line with our previous reports describing the immunogenic potential of Rv2958c, Rv2957 and Rv0447c based on human T-cell assays, in terms of cellular immune activation.

Figure 4. Vaccine efficacy assessment with BCG priming and KI-TB antigens without GLA-SE adjuvant. Mice were primed with BCG and given two boosts with KI-TB antigens 3 and 6 weeks later, without GLA-SE adjuvant. 3 weeks after the second boost (9 weeks post BCG priming), mice were challenged with 100-200 CFU of virulent M. tb H37Rv via the aerosol route. PBS-vaccinated mice were used as negative controls. At 6 and 12 weeks post challenge, the mice were sacrificed, and the lungs and spleens were removed for CFU assessment. Shown are the medians of 4-6 mice per group (Mann-Whitney T-test of medians; "p < 0.05; "p < 0.01).
Figure 5. Vaccine efficacy assessment with BCG priming and KI-TB antigens with GLA-SE adjuvant. Mice were primed with BCG and given two boosts with KI-TB antigens 3 and 6 weeks later, with GLA-SE adjuvant. 3 weeks after the second boost (9 weeks post BCG priming), mice were challenged with 100–200 CFU of virulent M. tb H37Rv via the aerosol route. PBS-vaccinated mice were used as negative controls. At 6 and 12 weeks post challenge, the mice were sacrificed, and the lungs and spleens were removed for CFU assessment. Shown are the medians of 5–6 mice per group (Mann-Whitney T-test of medians; *p < 0.05; **p < 0.01).

Conclusion

We show that boosting BCG priming with M. tb proteins involved in cell wall biosynthesis (Rv2958c, Rv2957 and Rv0447c) allows for improved control of M. tb burden in the lungs of mice compared to BCG alone. Furthermore, our results suggest that the M. tb antigens can be administered without an adjuvant due to the in-built immunogenic potential of the proteins themselves. Thus, we present evidence for further development of the KI-TB antigens (Rv2958c, Rv2957 and Rv0447c) in a heterologous prime-boost vaccination strategy with BCG.

Conflict of interest

The authors declare no conflicts of interest.

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