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Improved protection in guinea pigs after vaccination with a recombinant BCG expressing MPT64 on its surface

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ABSTRACT

The lack of an efficient vaccine against tuberculosis is still one of the major problems threatening global human health. In previous work we showed that expression of the protective antigen MPT64 on the surface of *Mycobacterium bovis* BCG, the only approved vaccine against tuberculosis, strongly improved its immunogenicity and protective potential in mice. In this work we demonstrate that the same recombinant strain is able to induce better protection than wild type BCG also in guinea pigs preventing *Mycobacterium tuberculosis* dissemination and lung pathology, making this strain a strong candidate for further testing.

1. Introduction

Tuberculosis (TB) is still one of the main scourges of human kind with about 9 million new cases of active disease and 1.5 million deaths each year [1]. Even though TB is a curable disease, treatment is long with patient compliance challenging, especially in developing countries where the infection is more diffused, boosting the emergence of strains resistant to the main anti-tubercular drugs [2]. The only approved vaccine against TB, *Mycobacterium bovis* BCG, protects against the most severe forms of extrapulmonary TB in infants, but its efficacy in preventing pulmonary disease in adults is poor [3]. For this reason, the development of a new vaccine against TB is one of the main challenges to stop the TB pandemic [4]. Given that it prevents severe disease in children, the use of BCG cannot be discontinued for ethical reasons, so any new vaccination strategy must incorporate BCG or an improved alternative e.g. recombinant BCG (rBCG) [5].

Since the mycobacterial surface is an excellent adjuvant, we hypothesized that expressing an antigen in its context could dramatically increase immunogenicity. Consequently, we developed a system to express proteins on the surface of BCG [6]. This is based on the PE domain of PE_PGRS33, recently identified as a functional domain able to drive the localization of protein sequences fused at its N-terminus on the mycobacterial outer membrane [7]. We used this system to express MPT64, a protective antigen of *Mycobacterium tuberculosis* normally absent in BCG, on its surface and demonstrated increased protection and immunogenicity compared to parental strain following vaccination and challenge of immunized mice with virulent *M. tuberculosis* [8]. Moreover, homologous boosting resulted in a heightened degree of protection of mice immunized with this $_{H}$PE-ΔMPT64-BCG strain [9].

In this paper we further characterize the protective potential of the $_{H}$PE-ΔMPT64-BCG strain in guinea pigs, by demonstrating improved protection of vaccinated animals from infection through surface expression of MPT64.

2. Materials and methods

2.1. Bacteria

The *M. tuberculosis* H37Rv (National Collection of Type Cultures (NCTC) 7416) challenge stock was generated from a chemostat grown to steady state in a defined medium which has been described previously [10].

2.2. Vaccination

Three groups of 8 Dunkin–Hartley guinea pigs, (specific pathogen-free, 250–300 g), obtained from a commercial supplier

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(Harlan, UK), were inoculated subcutaneously with 5×10^4 CFU of $_H$PE-ΔMPT64-BCG (rBCG expressing the PE_MPT64 chimera on its surface) [8] or BCG Danish 1331 (Statens Serum Institute, Copenhagen, Denmark), or were unvaccinated. Individual animals were identified using subcutaneously implanted microchips (PLEXX BV, The Netherlands). Guinea pig experimental work was conducted according to UK Home Office legislation for animal experimentation and was approved by the local ethical review process.

2.3. Aerosol challenge

Animals were infected with a low aerosol dose (10–50 CFU retained in the lung) of *M. tuberculosis* H37Rv 12 weeks after vaccination using a Henderson apparatus in conjunction with the AeroMP (Biaera) control unit as previously described [11,12]. The aerosol was generated from a water suspension containing 5×10^6 CFU/ml.

2.4. Necropsy and bacteriological analysis

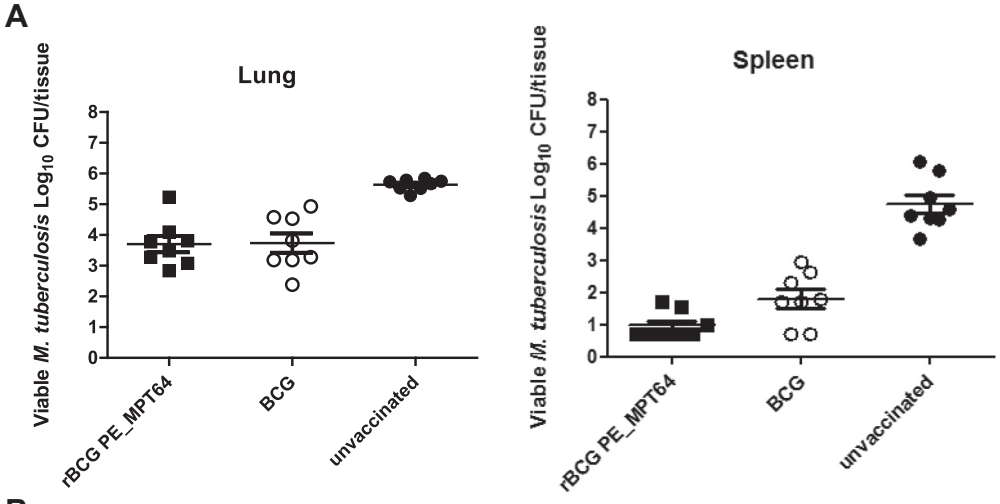
At 4 weeks post challenge, guinea pigs were killed humanely by intraperitoneal injection of pentabarbitone (Euthatal) and a post-mortem examination performed immediately. For each animal, the left middle and left and right cranial and the right caudal lobes were placed in one sterile container for bacteriology and the remaining lobes placed in 10% neutral-buffered formalin for histological examination. Tissues for determination of CFU were homogenized in 5 ml of sterile distilled water using a rotating blade macerator system (Ystral, UK). Viable counts were performed by plating 100 µl aliquots of serial dilutions on of the macerate onto Middlebrook 7H11 + OADC agar (BioMerieux, UK). Bacterial load in lungs and spleen (CFU/tissue) of each group of animals was compared to identify statistically significant differences between the groups.

2.5. Histopathological examination

Formalin-fixed samples were processed to paraffin wax, cut at 3–5 µm, and stained with haematoxylin and eosin. The nature and severity of the lesions in lungs and spleen was assessed blind by a veterinary pathologist using a subjective scoring system. Briefly, for the spleen, a score was assigned based on number and size of lesions, and presence of necrosis and calcification. For the lung, each lobe was assigned a score as follows: 0-normal; 1-very few or small lesions, 0–10% consolidation; 2-few or small lesions, 10–20% consolidation; 3-medium sized, 20–33% consolidation; 4-moderate sized lesions, 33–50% consolidation; 5–50–80% consolidation; extensive pneumonia; >80% consolidation; plus number of foci of necrosis. Scores from each lobe were combined. A mean score from lung lobe and from spleen was calculated for each group. Group mean differences were compared to subcutaneous BCG Danish and unvaccinated control groups. A reduction in consolidation, foci of necrosis, and foci of calcification (spleen) in the vaccinated animals when compared with the control groups was considered a protective effect of the vaccine. However, the scores are a product of a subjective scoring system and, therefore, are not regarded as numerical data suitable for statistical analysis.

2.6. Statistics

Statistical analysis was performed with Minitab software version 15. The CFU data were analyzed by non-parametric Mann–Whitney test comparisons to compare median values of the



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Vaccine	tissue	Mann-Whitney test P-value		Efficacy
		Vs. BCG	Vs. unvaccinated	
rBCG PE-MPT64	Lung	0.9163	0.0002	Equivalent to BCG
	Spleen	0.032	0.0008	Better than BCG

Fig. 1. (A) Determination of CFU in *M. tuberculosis*-infected guinea pigs. CFU were determined in lung (left) and spleen (right) homogenates derived from *M. tuberculosis*-infected guinea pigs that were unvaccinated (closed circle) or vaccinated with either BCG (open circle) or $_H$PE-ΔMPT64-BCG (closed square) as indicated on the x-axis. Protective efficacies are expressed as total $\log_{10}$ bacterial counts/tissue. Groups included eight guinea pigs. Each symbol represents one guinea pig. (B) Table summarising the efficacy of $_H$PE-ΔMPT64-BCG. CFU data were analyzed by non-parametric Mann–Whitney test comparisons to compare median values of the vaccine groups with either the unvaccinated or BCG control groups. A *P* value of <0.05 was considered significant.