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Characterization of W-Beijing isolates of *Mycobacterium tuberculosis* from the Western Cape

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ABSTRACT

The purpose of this simple study was to characterize a panel of clinical isolates of *Mycobacterium tuberculosis* obtained from the Western Cape region of South Africa where new clinical vaccine trials are beginning, in the low dose aerosol guinea pig infection model. Most of the strains tested grew well in the lungs and other organs of these animals, and in most cases gave rise to moderate to very severe lung damage. We further observed that the current BCG vaccine was highly protective against two randomly selected strains, giving rise to significantly prolonged survival.

1. Introduction

The global epidemic of disease caused by *Mycobacterium tuberculosis* shows no signs of abating, and is particularly serious in Sub-Saharan Africa, much of it driven by the HIV co-epidemic [1]. The seriousness of the situation, in part at least, reflects the large range of the efficacy of the current BCG vaccine [2,3] and the increasing drug resistance of many strains of *M. tuberculosis* [4–6].

The past decade has seen strenuous efforts to develop new more effective vaccines against tuberculosis, and the establishment of a pipeline of new candidates. Leading this list are recombinant BCG, a prime boost strategy using BCG and a recombinant vaccinia virus, and a fusion protein candidate delivered in a potent new adjuvant [7–9].

Clinical trials of new tuberculosis vaccines have started to be conducted at various sites, including in the Western Cape of South Africa. As yet however none of the current lead candidates have been tested against the newly emerging high virulence clinical isolates that represent the heart of the problem in this area. In this regard in fact, a recent expert panel suggested [2] that new candidates entering trials should first be tested against representative local isolates, but this has not been done yet. This is far from trivial, given our recent observations that many newly emerging strains (particularly W-Beijing strains) are of extremely high virulence in small animal models [10,11]. These models also reveal that these clinical strains, unlike the laboratory strains used to screen vaccines, seem to be able to elicit a much broader T cell response including potentially high levels of Foxp3+ regulatory T cells and IL-17 secreting cells [12,13], which has the potential to interfere with vaccine efficacy [12].

In this study, we show that a panel of representative clinical isolates obtained from patients attending clinics in the Western Cape region of South Africa mostly grow very well in guinea pigs exposed to low dose aerosol infection, and rapidly caused extensive to severe lung damage. Encouragingly however, prior vaccination with BCG was highly effective against two strains tested (picked randomly), reducing the lung burden and significantly extending animal survival. However if this represents a general trend for strains from this region it suggests that achieving an improvement by a new vaccine over the existing BCG vaccine may be difficult.

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Table 1
Clinical strains used in this study.

Isolate nr.	Strain family	Isoniazid resistance	Rifampicin resistance	Resistance pattern	rpoB
R5727	X-Family (DRF150)	S	R	Rifampicin mono resistant	+S/526TAC
R3239	Atypical Beijing	S	R	Rifampicin mono resistant	+S/526TAC
TT372	Atypical Beijing	S	S	Sensitive	
R5688	LAM3	S	R	Rifampicin mono resistant	+S/526CTC
R2139	S family	S	R	Rifampicin mono resistant	+S/526TAC
SAWC1063	S family	S	S	Sensitive	
R3180	Typical Beijing	S	R	Rifampicin mono resistant	+S/531TTG
SAWC3382	Typical Beijing	S	S	Sensitive	
R2135	Typical Beijing (cluster R220)	S	R	Rifampicin mono resistant	+S/531TTG
SAWC954	Typical Beijing (cluster R220)	S	S	Sensitive	

This hypothesis is discussed further below in the context of the disappointing results very recently reported regarding the Phase II trial of the MVA85A vaccine candidate [14].

2. Materials and methods

2.1. Animals

Female outbred Hartley guinea pigs (~450–500 g in weight) were purchased from the Charles River Laboratories (North Wilmington, MA) and held under barrier conditions in a biosafety level III animal laboratory. The specific-pathogen-free nature of the guinea pig colonies was demonstrated by testing sentinel animals. All experimental protocols were approved by the Animal Care and Usage Committee of Colorado State University and comply with NIH guidelines.

2.2. Experimental design

Ten clinical strains from the Western Cape in South Africa were selected from an existing strain collection, which is maintained in the Division of Molecular Biology and Human Genetics at Stellenbosch University (Table 1). Six of these strains, designated R3180, R2139, R3239, R5727, R5688, and R2135 were rifampicin mono-resistant by culture and have a prominent mutation in the *rpoB* gene. Four strains designated SAWC954, SAWC1063, SAWC3382 and TT372 were completely drug sensitive with a wild type *rpoB* gene. These strains represent the most prominent drug sensitive and drug resistant genotypes in the region and are described in detail elsewhere [15–20]. All strains were grown in 7H9 broth containing 0.05% Tween 80 and OADC. When a strain had an OD_{600} reading of 0.60–1.00 it was bottled, frozen, and then titered. Thawed aliquots of frozen cultures were diluted in sterile saline to the desired inoculum concentration of 1×10^6 cfu/ml. The infection inoculum was determined for all the bacterial strains tested by plating serial dilutions of inoculum on nutrient 7H11 agar containing 10 μg/ml cycloheximide and 50 μg/ml of carbenicillin to prevent contamination. Colonies were counted after 3 weeks incubation at 37 °C. No significant differences in terms of the infection dose were seen among any of the strains tested. A Madison chamber aerosol generation device was used to expose the animals to *M. tuberculosis*. This device was calibrated to deliver approximately 10–20 bacilli into the lungs [21].

2.3. Bacterial load

On days 30 and 60 after aerosol exposure, the bacterial load in the right cranial lung lobe, spleen, and medistinal lymph nodes were determined by plating serial dilutions of tissue homogenates on nutrient 7H11 agar containing 10 μg/ml cycloheximide and 50 μg/ml of carbenicillin. Colonies were counted after 3 weeks of incubation at 37 °C and data expressed as $\log_{10}$ cfu.

2.4. Histological analysis

Right caudal lung lobes from each guinea pig were fixed with 4% paraformaldehyde in phosphate-buffered saline. Paraffin-embedded sections from these tissues were cut to 5 μm, mounted on glass slides, de-paraffinized, and stained using hematoxylin and eosin.

2.5. BCG vaccination

Guinea pigs were vaccinated intradermally with 1×10^4 *Mycobacterium bovis* BCG, strain Pasteur, or mock vaccinated with saline. *M. bovis* BCG was grown in Proskauer-Beck broth. Animals were challenged by aerosol exposure as above 6 weeks later.

2.6. Kaplan–Meier survival assay

Survival of animals was monitored by weighing and observation based on a modified Karnofsky scale. A guinea pig was euthanized if the animal showed extensive labored breathing, was lethargic, had a matted or scruffy coat, and if the weight loss was greater than 20% of weight at the time of challenge.

3. Results

3.1. Course of infection in target organs

The capacity of each isolate to grow in guinea pigs after deposition of approximately 20 bacilli into the lungs is shown in Fig. 1. All ten strains grew progressively in the lungs, reaching between 5 and 7-log in terms of the bacterial burden. Thereafter the growth of the infection was contained, although only one strain, the rifampicin mono-resistant strain R2139, showed evidence of any significant clearance. All ten strains showed evidence of extra-pulmonary dissemination to the spleen and draining lymph nodes. In the latter organs the bacterial load diminished, probably reflecting the increasingly severe lymphadenopathy and tissue destruction, as we have previously described [22].

3.2. Lung pathology

In all cases, infection with the panel strains caused progressive lung damage due to granulomatous inflammation (Figs. 2–4). By day-60 lung consolidation was extensive, particularly in the cases of R3180 and R5737, with moderate to severe consolidation and damage also seen in animals infected with TT372, SAWC3382, R3239, R5688, and SAWC954. More moderate lung damage was seen in guinea pigs infected with SAWC1063, R2135, and R2139.