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The rLVS Δ*capB*/*iglABC* vaccine provides potent protection to Fischer rats against aerosol challenge with multiple virulent *Francisella tularensis* strains

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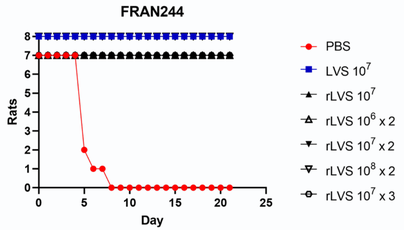
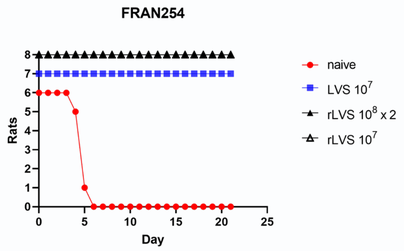
Introduction

Francisella tularensis is one of several biothreat agents for which a licensed vaccine is needed. To ensure vaccine protection is achieved across a range of *F. tularensis* strains, we generated and characterized a panel of isolates to be utilized as challenge strains in ongoing efforts to develop an effective vaccine against pneumonic tularemia [1]. A promising tularemia vaccine candidate is rLVS Δ*capB*/*iglABC* (rLVS), which is the LVS strain with a highly attenuating deletion in the *capB* gene and expresses a fusion protein (IglABC) comprising immunodominant epitopes of Type VI Secretion System proteins IglA, IglB, and IglC encoded by Francisella Pathogenicity Island genes [2, 3].





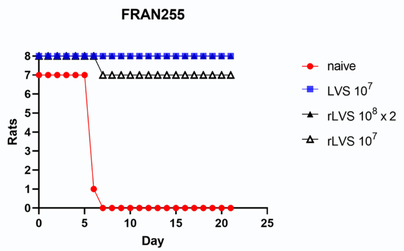


Figure 1. Survival data from rLVS vaccinated rats following whole body aerosol challenge with F. tularensis. Groups of Fisher rats were vaccinated with the corresponding dose/s of rLVS. The control groups received either PBS or LVS parent strain. Three-weeks after the last vaccination, the rats were challenged by aerosolization with (A) FRAN244/Schu S4, (B) FRAN254, or (C) FRAN255.

Methods

Vaccination. Fisher rats (8/group) were immunized subcutaneously 1-3 times at 3-week intervals with rLVS at various doses (10^6-10^8 CFU) **(Tables 1 & 2).** As positive and negative controls for these vaccine studies, separate groups of rats received the LVS parent strain at a single dose (10^7 CFU) or PBS alone.

Aerosol Challenge. Rats were challenged by whole body aerosolization with a high dose of the Type A strain Schu S4/FRAN244 at 72 LD_{50}, a clinically obtained Type B strain (FRAN255) at 73 LD_{50}, or a tick derived Type A strain (FRAN254) at 233 LD_{50}. Following challenge, the rats were monitored for survival for 21 days. Blood was collected from the rats pre- and post-vaccination and from survivors at the end of the study to measure antibody levels against irradiated Schu S4 (Type A) or FRAN255 (Type B) antigens. In addition, at the end of the study, vaccinated survivors were examined for lung, liver, and spleen for pathologic analysis.

Table 1. Vaccine strategy to test protection against Schu S4/FRAN244 in rats.

Vaccine Strain	Dose (CFU)	Dose (CFU)	Dose (CFU)
PBS- naive	-	-	-
LVS	-	-	$1.24x10^7$
rLVS	-	-	$1.21x10^7$
rLVS	-	$2.71x10^6$	$1.04x10^6$
rLVS	-	$1.15x10^7$	$1.21x10^7$
rLVS	-	$1.46x10^8$	$1.42x10^8$
rLVS	$1.92x10^7$	$1.15x10^7$	$1.21x10^7$

Table 2. Vaccine strategy to test protection against FRAN254 and FRAN255 in rats.

Vaccine Strain	Dose (CFU)	Dose (CFU)
PBS- naive	-	-
LVS	-	$5.2x10^7$
rLVS	-	$5.8x10^7$
rLVS	$9.7x10^7$	$8.8x10^7$

Results

Protection studies. For the initial study, the ability of rLVS to protect rats against the Schu S4/FRAN244 strain was determined using the vaccine strategy listed in Table 1. Following exposure to 72 LD_{50} of Schu S4, all rats receiving a vaccine (either LVS or rLVS) survived the challenge (Fig. 1A). In contrast, all rats in the naïve group succumbed to infection by Day 8.

Based upon the protection provided by rLVS against the Schu S4 strain, we proceeded to challenge vaccinated rats with the down selected *F. tularensis* panel strains (FRAN254 and FRAN255). In addition, the vaccination strategy for rLVS was modified to consist of a single vaccination at ~10^7 CFU or two vaccinations at ~10^8 CFU each (Table 2). The controls for this study were again only PBS (naïve) or LVS parent with a single vaccination at ~10^7 CFU. Three weeks after the final vaccination, the rats were challenged by whole body aerosolization with FRAN254 (233 LD_{50}) or FRAN255 (73 LD_{50}). All rats receiving either LVS or rLVS survived the high challenge with FRAN254 (Fig. 1B). In contrast, all rats in the naïve group succumbed to infection by Day 6. For those rats exposed to aerosols of the Type B strain FRAN255, one of the rats vaccinated singularly with the 10^7 CFU dose of rLVS succumbed to infection on Day 7. The remaining vaccinated rats with either LVS or rLVS survived to the end of the study at Day 21 (Fig. 1C).

Immune Analysis. The total serum IgG response was assessed from the rats prior to and after the final vaccination per the various strategies (Tables 1 & 2). For the Schu S4/ FRAN244 experiment, the highest total IgG responses directed against *F. tularensis* were observed in mice vaccinated with rLVS x 2 (10^8 CFU) followed by rats vaccinated with rLVS x 3 (10^7 CFU) (Table 3). For the following experiments with the additional *F. tularensis* challenge stains and down-selected vaccination strategy, rats vaccinated with a double dose of rLVS had similar IgG responses to antigens directed against both Type A and B strains of *F. tularensis* (Table 4).

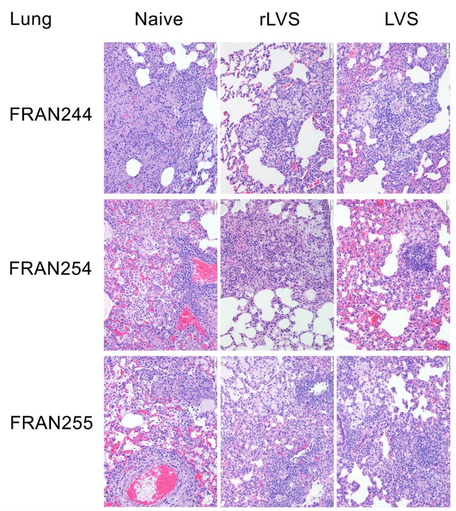


Figure 2. Histopathology comparison of lungs from vaccinated FRAN244, FRAN254, and FRAN255 infected rats.

Histopathology. For all of the vaccinated (LVS or rLVS) rats surviving challenge, no significant pathological differences within the lungs were observed between any of the survivors. Lung from every group that was reviewed microscopically had similar histopathologic changes with multifocal areas of alveolar inflammation consisting of macrophages, lymphocytes, and plasma cells; severity ranged from mild to moderate (Fig. 2). The lesions observed in all animals were considered to be resolving and were a consequence of aerosolized *F. tularensis* infection. This observation was in contrast to unvaccinated rats which had succumbed to infection. These rats showed alveolar necrosis and damage, more severe and widespread inflammation (moderate to marked), and hyperplasia of bronchus associated lymphoid tissue (Fig. 2).

Table 3. Total serum IgG response to vaccinated rats prior to challenge with FRAN244/Schu S4

Vaccine	N	Antigen	Geo Mean	GSE
PBS	8	Type A	2,012	1.58
LVS (10^7)	8	Type A	35,812	1.67
rLVS (10^7)	7	Type A	13,125	1.28
rLVS x 2 (10^6)	7	Type A	33,074	1.38
rLVS x 2 (10^7)	8	Type A	39,170	1.33
rLVS x 2 (10^8)	8	Type A	130,972	1.33

Table 4. Total serum IgG response among vaccinated rats prior to challenge with FRAN254 (Type A) & FRAN255 (Type B)

Vaccine	Antigen	Geo Mean	GSE
FRAN254 (Type A)			
PBS	Type A	3,335	1.95
	Type B	2,908	2.08
LVS	Type A	235,640	1.25
	Type B	291,130	1.28
rLVS x 2	Type A	282,842	1.29
	Type B	259,881	1.28
rLVS	Type A	103,439	1.22
	Type B	112,579	1.31
FRAN255 (Type B)			
PBS	Type A	4,733	1.31
	Type B	5,570	1.58
LVS	Type A	317480	1.24
	Type B	388,997	1.42
rLVS x 2	Type A	377,549	1.21
	Type B	282,842	1.25
rLVS	Type A	92,245	1.28
	Type B	97,676	1.20

Conclusion and Discussion

Currently, the U.S. biodefense community is lacking an approved FDA vaccine to prevent tularemia. There are some safety concerns and the potential of reversion associated with the unlicensed LVS. However, a modified version of LVS (rLVS Δ*capB*) has the potential to be used as a vaccine platform to express immunogenic proteins against multiple pathogens in addition to *F. tularensis* and was demonstrated to be much less virulent than the parent LVS in mice. The rLVS Δ*capB*/*iglABC* vaccine was shown to be highly protective for mice against numerous challenge routes with the Schu S4 strain of *F. tularensis*. Here, we further demonstrated the potential of rLVS as a vaccine in the rat model of tularemia by whole body aerosol challenge with the prototype Schu S4 strain and additional strains of *F. tularensis*. These results demonstrate that rLVS Δ*capB*/*iglABC* is able to provide potent protection against aerosol challenge with both Type A and Type B *F. tularensis* strains and should be considered for further analysis as a future tularemia vaccine.

References

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