19 March 2007

TARGET Report – Study performed by Drs. Lan Ly and David McMurray; histopathology and report prepared by Dr. Paul Converse

Experiment in guinea pigs using mutants obtained from Dr. Beata Wolucka

Strains

1. H37Rv (TAMU, Dr. McMurray)
2. H37Rv (IPB, Institut Pasteur, Brussels, Dr. Wolucka)
3. H37Rv Δ*Rv1771*
4. H37Rv Δ*Rv1771* complemented

Goals:

- Assess bacterial burden on day 1 after infection in entire lung (5 lobes)
- Assess bacterial burden on week 4 after infection in lung and spleen
- PPD skin test at 4 weeks after infection
- Lung histopathology at 4 weeks after infection

Day 1 results after aerosol exposure to 2 x 10^7 bacilli/ml

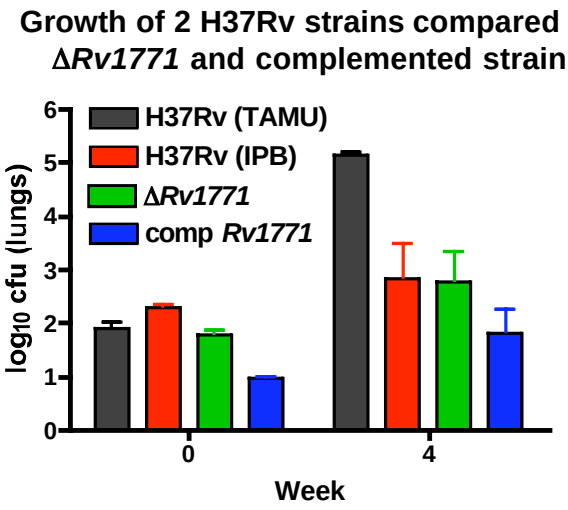
The entire lung (5 lobes) was homogenized in 9 ml of sterile saline and 2 ml. were plated onto 20 plates to determine implantation

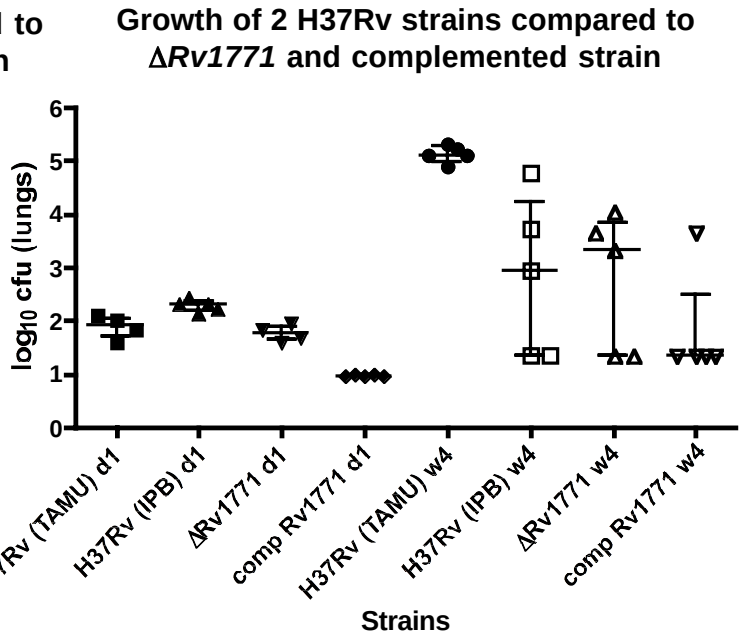
Strain	**Mean Total CFU in lung (N=5)**	**Log_{10} CFU ± SEM**
H37Rv (TAMU)	85 (one sample contaminated)	1.89 ± 0.11
H37Rv (IPB)	202	2.29 ± 0.05
H37Rv Δ*Rv1771*	62.5 (one sample contaminated)	1.77 ± 0.08
H37Rv Δ*Rv1771* complemented	9.4 (3 animals below detection limit)	0.97 ± 0.01

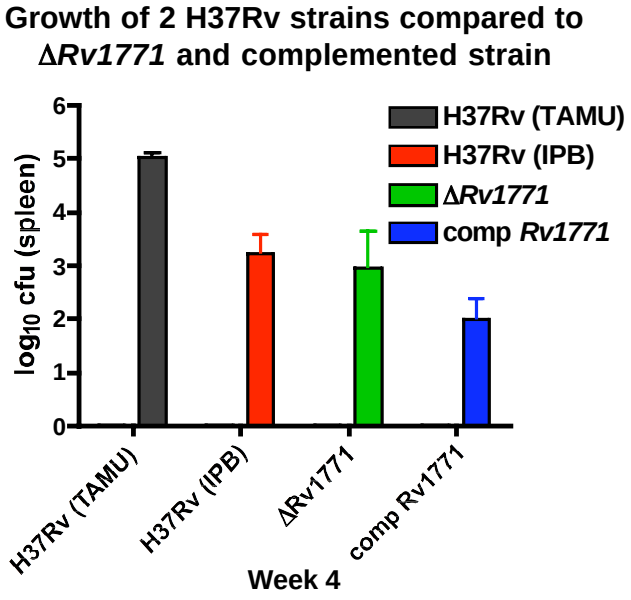
Week 4 results after aerosol exposure to 2 x 10^7 bacilli/ml

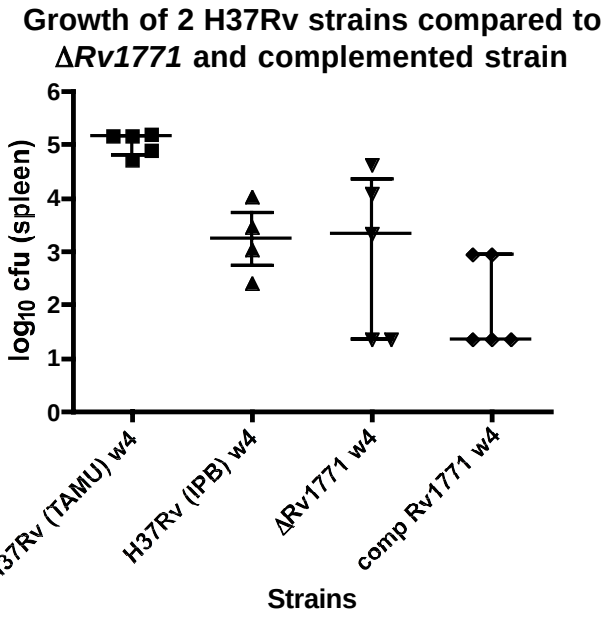
PPD skin tests performed; Lungs and spleens were homogenized for bacteriology and remaining tissues were taken for histopathology.

Strain	**Log_{10} cfu in lung (N=5) ± SEM**	**Log_{10} cfu in spleen (N=5) ± SEM**
H37Rv (TAMU)	5.13 ± 0.07	5.02 ± 0.09
H37Rv (IPB)	2.83 ± 0.67 (2 animals below detection limit)	3.22 ± 0.34 (specimen from 1 animal contaminated)
H37Rv Δ*Rv1771*	2.75 ± 0.58 (2 animals below detection limit)	2.95 ± 0.68 (2 animals below detection limit)
H37Rv Δ*Rv1771* complemented	1.81 ± 0.46 (4 animals below detection limit)	1.99 ± 0.39 (3 animals below detection limit)





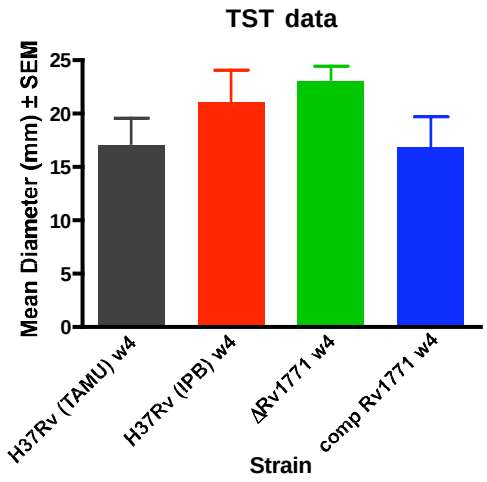




Both the parent and deletion mutant strain appeared to have no defect in implantation. The complemented mutant implanted much less well. Growth of the parent and mutant strains was much less than that of the H37Rv strain from TAMU. At 4 weeks, there is little variability in the TAMU strain but much more is apparent in the other strains. The bar graphs show means ± SE. The scatter plots show median and interquartile range.

PPD skin tests at 4 weeks

Strain	Week 4 (mean ± SEM)
H37Rv (TAMU)	17 ± 2.55
H37Rv (IPB)	21 ± 3.02
H37Rv Δ*Rv1771*	23 ± 1.46
H37Rv Δ*Rv1771*	16.8 ± 2.80



Lung Histopathology

Lungs were evaluated in terms of number of granulomas, number of low power fields per section, and by a subjective evaluation score (1-4) of the condition of the lung considering the number and size of the granulomas as well as the extent of inflammatory infiltration. Two sections were evaluated for each strain. The wild-type strain had the most lesions and highest score. Portions of lungs with lesions are shown in the micrographs [20x].

GP #	Group	Strain	# granulomas	#fields	Score (1-4)
827	5	TAMU H37Rv	3.25	1	2.5
832	5	TAMU H37Rv	5.5	1	3.0
803	6	IP-B H37Rv	1	2.5	1.25
809	6	IP-B H37Rv	1.5	1.5	1.5
808	7	ΔRv1771	0	2	1.1
837	7	ΔRv1771	1	1.5	1.5
816	8	Comp ΔRv1771	0	1	1.1
834	8	Comp ΔRv1771	0	2	1.5