TARGET Report – prepared by Paul Converse, Ph.D. and Eric Nuermberger, M.D.
Experiment in C57BL/6 and BALB/c mice using strains obtained from Dr. David Carroll.
Study performed by Drs. Eric Nuermberger and Jacques Grosset, Johns Hopkins University.

Strains

1. H37Rv (from JHU)
2. H37Rv Δ*treS*
3. H37Rv Δ*treS* complemented
4. H37Rv Δ*otsA*
5. H37Rv Δ*otsA* complemented

} all mutants from Dr. Brian Robertson

Goals:

- Mouse passage of all strains x 2.
- PCR confirmation of all mutant strains before and after passage.
- Phenotype confirmation of all mutant strains after passage.
- Quantitative CFU counts after iv infection of 10-14 wk old C57Bl/6 mice with 10^6 bacilli at the time points indicated in the table below.
- Gene expression in flash frozen 1/2 organs from these mice at each time point.
- Time-to-death in 4-6 wk old BALB/c mice after same iv infection.

Hypothesis: The mutant tubercle bacilli will have an attenuated phenotype and behave like persistence mutants such as Δ*icl*.

Experimental Scheme*

	Time point and # of C57Bl/6 mice sacrificed							
Mtb strain	D1	W2	W4	W8	W12	W16	W24	Total
H37Rv	3	5	5	5	5	5	5	33
Δ*otsA*	3	5	5	5	5	5	5	33
Δ*treS*	3	5	5	5	5	5	5	33
cΔ*otsA*	3	5	5	5	5	5	5	33
cΔ*treS*	3	5	5	5	5	5	5	33
Total	**15**	**25**	**25**	**25**	**25**	**25**	**25**	**165**

* 10 4-6 wk old BALB/c mice also were infected and observed for mortality.

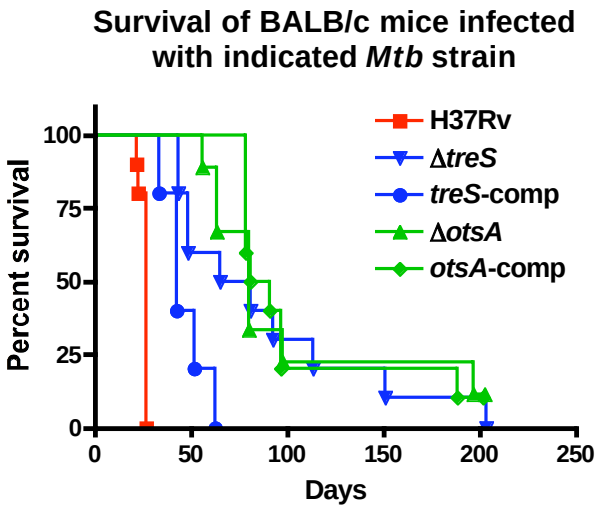
Day 1 results after intravenous infection

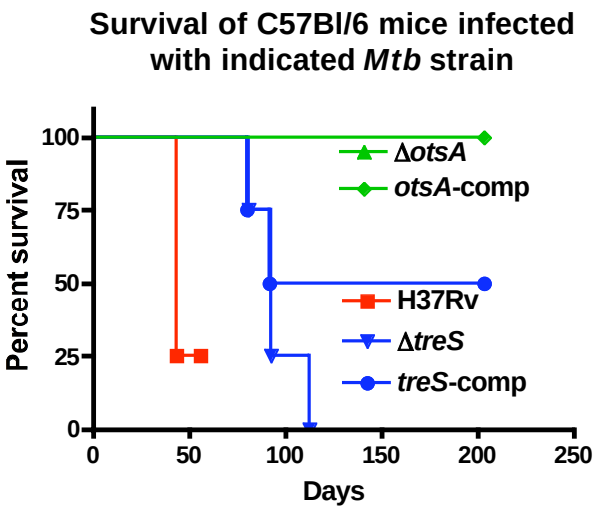
The entire lung and spleen were each homogenized in 2.5 ml of sterile saline and serial dilutions were plated to determine implantation.

Strain	Log_{10} CFU/lung ± SD (N=5)	Log_{10} CFU/spleen ± SD (N=5)
H37Rv	5.00 ± 0.11	6.18 ± 0.15
H37Rv Δ*treS*	4.45 ± 0.26	6.10 ± 0.30
H37Rv Δ*treS* complemented	4.70 ± 0.18	6.01 ± 0.20
H37Rv Δ*otsA*	4.51 ± 0.43	5.82 ± 0.09
H37Rv Δ*otsA* complemented	4.90 ± 0.31	6.09 ± 0.15

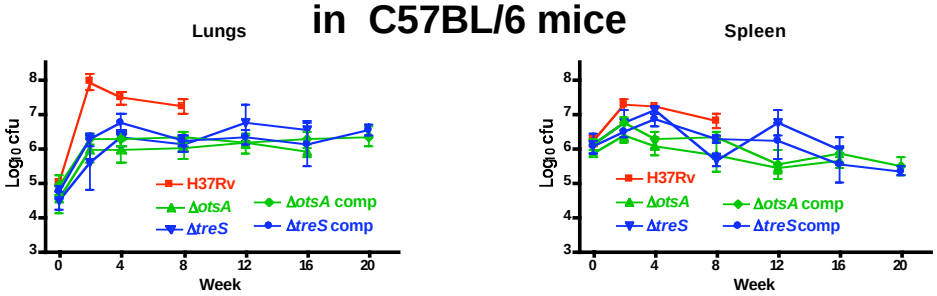
The number of organisms in the spleen on the day after infection was very similar between strains. The difference between strains was slightly greater in the lungs. Both deletion mutants were about 0.5 log_{10} CFU lower than the H37Rv wild-type. However, the differences between strains were not statistically significant (p=0.1487 by one way ANOVA). Overall, the number of CFU implanted in the spleen appeared similar to that reported by Murphy *et al*. who reported day 10, but not day 1, counts (Murphy HN, Stewart GR, Mischenko VV, Apt AS, Harris R, McAlister MS, Driscoll PC, Young DB, Robertson BD. The OtsAB pathway is essential for trehalose biosynthesis in *Mycobacterium tuberculosis*. J Biol Chem. (2005) 280:14524-9. PMID: 15703182). However, the number implanted in the lungs was at least one log_{10} higher in the current experiment. The CFU counts obtained over the full course of the experiment are presented and discussed on the following pages.

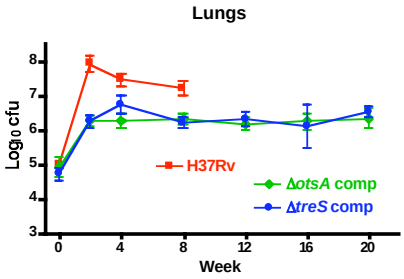
Survival was also assessed in 4-6 week-old BALB/c mice as well as 14 week-old C57BL/6 mice, as shown below. Both deletion mutants and the *otsA* complemented strain were significantly less virulent than the *treS* complemented strain (p<0.005 by log rank test) and H37Rv (p<0.0001) and the *treS* complemented strain was significantly less virulent than H37Rv (p<0.0001). The remaining strains could not be distinguished from one another. The C57BL/6 mice survived better than the BALB/c mice, although the difference was less striking for H37Rv. In addition to a strain difference, differences in the age of the mice cannot be ruled out as a contributing factor in the improved survival of the C57BL/6 mice.

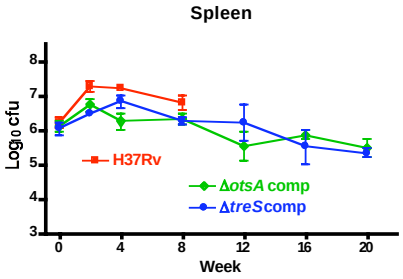


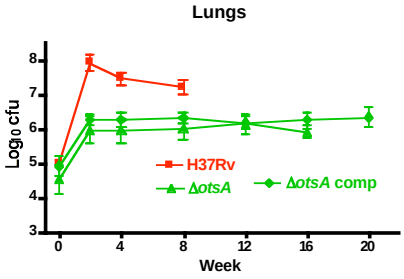


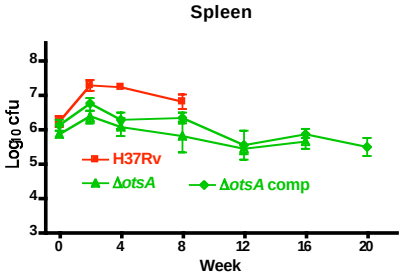
Growth of *M. tb* H37Rv wild-type and trehalose mutants in C57BL/6 mice

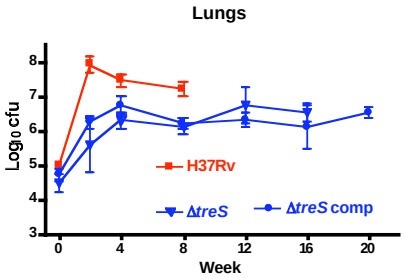


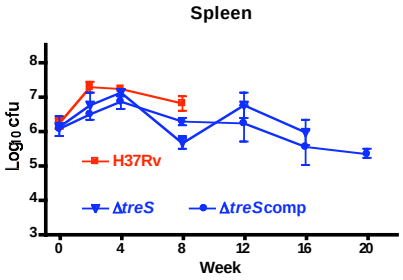


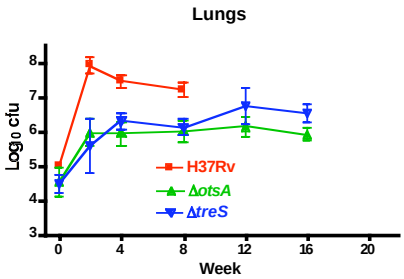


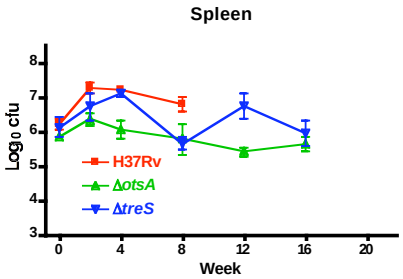












Bacterial replication through week 16:

The lung and spleen CFU counts demonstrated that all mutants were attenuated compared to H37Rv. This attenuation was most marked in the lungs. Growth in the spleen was not very different at most time points except for the Δ*otsA* mutant. There were not significant differences between each trehalose mutant and its complemented strain but the spleen CFU counts for the Δ*otsA* mutant were lower than those for the Δ*treS* mutant at 2 of the 5 time points after Day 1. Week 24 data are not reported because there were no surviving mice among those infected with H37Rv, Δ*otsA*, or Δ*treS*. There were 2 mice surviving at week 20 among those infected with the *treS* complemented ($\log_{10}$ CFU ± SD: 6.50 ± 0.15 in lungs and 5.31 ± 0.13 in spleen) and three mice infected with the *otsA* complemented strain ($\log_{10}$ CFU ± SD: 6.32 ± 0.27 in lungs and 5.45 ± 0.27 in spleen). Isolates obtained from the week 16 and 20 time points were tested for susceptibility to kanamycin and hygromycin to confirm the identity of the infecting strains.

Conclusions

From these data, we conclude that the Δ*treS* mutant is attenuated in lethality, but not proliferation, compared to the complemented strain. On the other hand, the Δ*otsA* mutant could not be distinguished from its complemented strain. Moreover, all mutants and complemented strains are attenuated for lethality and proliferation compared to the JHU H37Rv control strain. It is possible that this H37Rv strain is more virulent than the parent strain in which the trehalose deletion mutants and their complements were generated.