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TARGET Report – prepared by Paul Converse, Ph.D. and Eric Nuermberger, M.D.
Experiment in BALB/c mice using strains obtained from Drs. Tom Shinnick and Mani Cheruvu.
Study performed by Drs. Eric Nuermberger and Jacques Grosset, Johns Hopkins University.

Strains

1. H37Rv
2. H37Rv Δ*Rv3083-3089*
3. H37Rv Δ*Rv3083-3089* complemented

Goals:

- Achieve matched implantation by using high and low aerosol doses of each strain
- Assess bacterial burden on day 1 after infection in lung
- Assess bacterial burden on weeks 2, 4, 8, and 16 after infection in lung and spleen
- Determine whole mouse and spleen weights at each time point
- Lung gross pathology and histopathology at weeks 2, 4, 8, and 16 after infection
- Survival in high dose infected mice

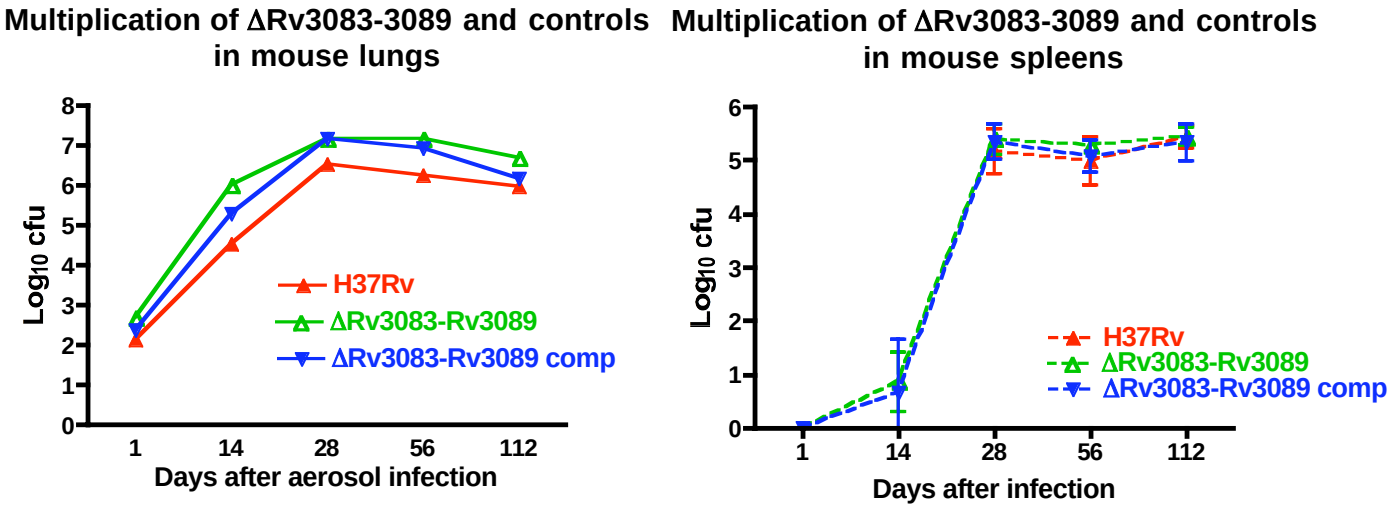
Day 1 results after aerosol infection

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

Strain	**Dose**	**Log_{10} CFU/lung ± SD (N=5)**
H37Rv	High	2.11 ± 0.13
	Low	1.35 ± 0.23
H37Rv Δ*Rv3083-3089*	High	3.26 ± 0.06
	Low	2.68 ± 0.05
H37Rv Δ*Rv3083-3089* complemented	High	2.38 ± 0.11
	Low	1.78 ± 0.15

Since the high dose H37Rv infected mice received a sub-lethal dose, time-to-death experiments were not pursued further. The best matches were with the high-dose H37Rv (2.11 ± 0.13), the low-dose deletion mutant (2.68 ± 0.05), and the high-dose complemented deletion mutant (2.38 ± 0.11).

Bacterial replication through week 16



From these data, we conclude that the H37Rv Δ*Rv3083-3089* mutant is not attenuated in the mouse aerosol model of tuberculosis. This mutant has, however, shown an attenuated phenotype in a human macrophage model of infection (Mani Cheruvu, Bonnie B. Plikaytis, Thomas M. Shinnick (2007) The acid-induced operon *Rv3083–Rv3089* is required for growth of *Mycobacterium tuberculosis* in macrophages. Tuberculosis **87**: 12–20. PMID: 16893682).

Although the level of implantation was too low to detect lethality with most of the strains, the high dose inoculum of the Δ*Rv3083-3089* mutant was implanted at a level (3.26 $\log_{10}$ cfu) where mortality was expected. Mice receiving this infectious dose had a median survival of 108 days and all of the mice in this group had succumbed by day 115. In our experience, mice typically succumb within 28-35 days after a similar infectious dose of our own H37Rv strain. However, that strain has been mouse-passaged multiple times and is probably more virulent than the parent strain used in these experiments in terms of bacterial multiplication and lethality.