TARGET Report – prepared by Paul Converse, Ph.D. and Eric Nuermberger, M.D.

Evaluation of growth and persistence of a *Mycobacterium tuberculosis* mutant with insertional inactivation of *lipY* in BALB/c mice

Experiment proposed by Dr. Laurent Kremer.
Mutant and complement generated by Gyanu Lamichhane, PhD.
Study performed by Drs. Eric Nuermberger and Jacques Grosset.

I. Passage and testing of TARGET mutant strains in mice.

Background: LipY (Rv3097; MT3181) is the only PE family protein to which a function has been assigned. It possesses triacylglycerol (TAG) hydrolase (lipase) activity. It is present only in the *M. tuberculosis* complex and *M. marinum*, two species capable of entering dormancy and to induce granuloma formation. The restricted distribution of this gene in a few pathogenic species suggests that it may participate in virulence and/or the immunopathogenesis of tuberculosis. Its localization to the surface of the organism suggests that the enzyme may interact with host macrophages and the immune system. Indeed, sera from TB patients exhibit a strong humoral response against LipY, indicating it is produced during infection. The *lipY* gene is induced under starvation conditions to utilize stored TAG as shown by and TAG utilization is drastically decreased under nutrient-deprived condition in a *lipY*-deficient mutant (Deb et al., 2006). Since TAGs have been shown to accumulate in dormant-like cultures of *M. tuberculosis*, perhaps as a source of carbon and energy in dormancy (Daniel *et al*., 2004), LipY may be responsible for the utilization of stored TAG during dormancy and reactivation of the pathogen. In addition, given the cell-surface exposure of LipY, it may also interact with the host cell and hydrolyze the host TAGs to the organism's advantage. The following experiment was designed to test whether a *lipY* mutant of *M. tuberculosis* would be affected in intracellular growth/survival or/and persistence in an animal model.

Experimental scheme:

	Time point and number of mice to sacrifice								
Strain	**D1**	**D14**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**Total**
H37Rv	5	5	5	5	5	5	5	5	**40**
CDC1551 (parent)	5	5	5	5	5	5	5	5	**40**
Tn *lipY*	5	5	5	5	5	5	5	5	**40**
Comp Tn *lipY*	5	5	5	5	5	5	5	5	**40**
Total	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**160**

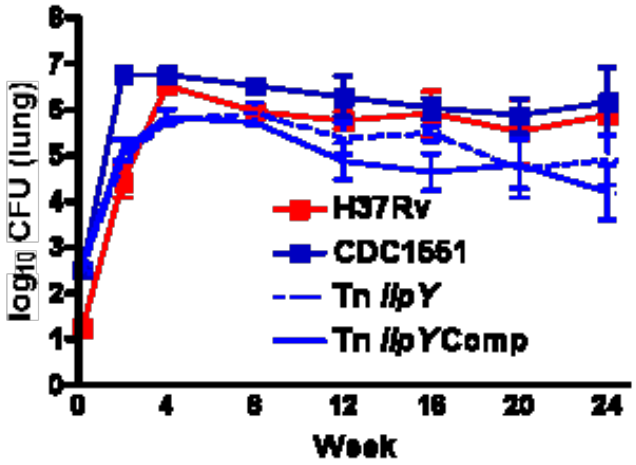
Methods: The transposon mutant strain in *Mtb* CDC1551 LipY from TARGET (JHU3097c-188) was passaged twice through mice. Dr. Kremer provided a plasmid encoding the gene with a hygromycin resistance marker for complementation. The complemented mutant was prepared by the TARGET Bacterial Genetics group and was

also mouse passaged. The identity of the strains was confirmed by Western blot and by drug susceptibility testing following mouse passage.

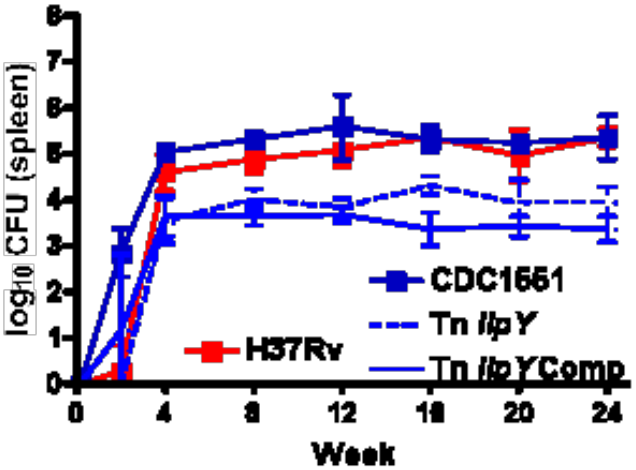
Aerosol infection of 6-week-old female BALB/c mice took place on April 1-2. Lung and spleen CFU counts were determined at the indicated time points.

Results: Lung and spleen CFU counts through 6 months post-infection are provided in the figures below. The parent, mutant and complemented strains were all implanted at approximately 2.5 log CFU in the lungs. The H37Rv control strain was implanted at approximately 1 log lower. The increase in lung CFU counts during the first 2 weeks was greatest for the 2 wild-type strains, followed by the 2 mutants. Whereas the parent strain counts reached a plateau by the second week, the lung counts for the other strains increased for the first month, consistent with expectations for strains implanted at lower doses or growing more slowly. After the first month, there has been a modest decline in counts for all strains that is perhaps greatest for the mutants. Spleen CFU counts reached a plateau between the 1st and 2nd months, which was proportional to the initial growth in the lungs, but the counts have remained stable after 3 months.

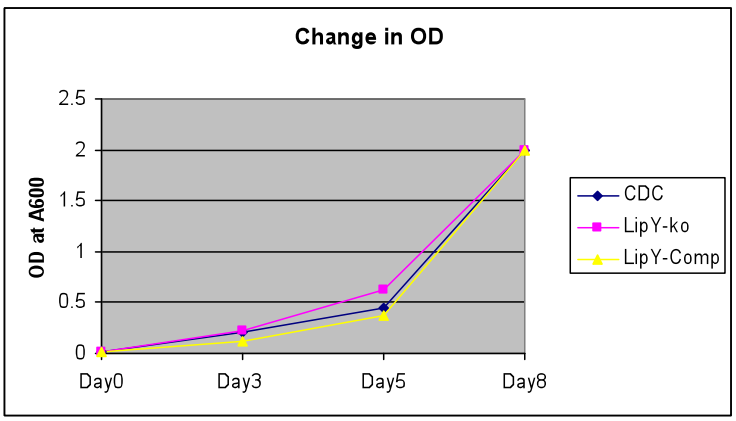
Multiplication in lungs of *lipY* transpon mutant its complement, and CDC1551 parent strain

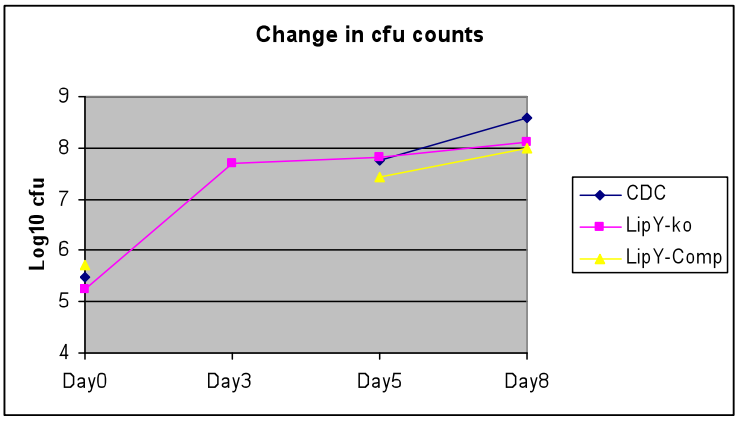


Dissemination to spleen of *lipY* transpon mutant its complement, and CDC1551 parent strain

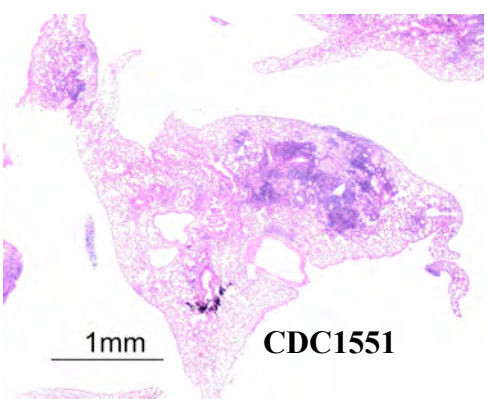


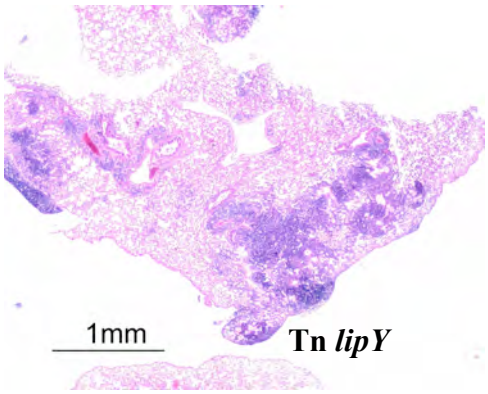
In vitro growth. Examinations were also made to assess any in vitro growth defect but none was found as shown below:

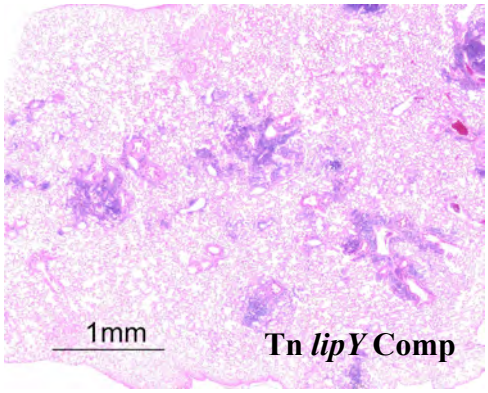




Histopathology. At 5 months, and again at 6 months (shown below), we saw no major differences in the histopathology of the lungs between the mutants and the wild-type.







<u>Conclusions</u>: The *lipY* mutant is attenuated in its growth in the lungs and spleens of mice, as is the complemented strain. The defect of the Tn mutant and its complement are primarily growth rather than persistence defects. The mutant has no in vitro growth defect compared to wild-type and there were no obvious differences in inflammatory pathology between the mutant and parental CDC1551 *M. tuberculosis* strain.