Investigating Prophylactic Respiratory Delivery of Active Bacteriophage D29 to Protect against Inhaled *Mycobacterium tuberculosis* H37Rv in Mice

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**Introduction:** Bacteriophage (phage) D29 is a parasitic virus of various mycobacteria, including *Mycobacterium tuberculosis (Mtb)*, which it is capable of lysing regardless of antibiotic resistance. While phage D29 may have little success penetrating granuloma, prophylactic respiratory delivery may allow it to bind to and lyse free bacteria in the alveoli.

**Aim:** Determine whether respiratory delivery of active phage D29 provides prophylactic protection against inhaled *M. tuberculosis* H37Rv aerosol in mice.

**Methods:** Female C57BL/6 mice were placed in a nose-only inhalation device and phage D29 aerosol was delivered. Within 30 minutes, the mice were placed in an aerosol chamber and either an ultra-low dose (~5-10 CFU) or a low dose (~20-100 CFU) of *Mtb* H37Rv aerosol was delivered. The lungs were extracted and homogenized for quantifying D29 and H37Rv.

**Results:** The number of PFU of phage D29 delivered to the lungs of the mice was approximately the same as the number of alveoli, indicating the target dose of an average of 1 PFU/alveolus was achieved. Ultra-low dose challenge (Figure 1) and low dose challenges resulted in significant reductions in *Mtb* bacterial levels.

**Conclusion:** Prophylactic respiratory delivery of phage D29 aerosol appears promising for protecting against primary infection with *Mtb* H37Rv aerosol. This finding may have implications for the development of products to provide extra protection to healthcare professionals treating active tuberculosis and to other individuals at high risk of exposure.
Figure 1: Pre-treatment with mycobacteriophage D29 aerosol significantly reduces pulmonary bacterial burden of inhaled *Mtb* H37Rv in mice.