Nebulized Bacteriophage D29 Provides Prophylactic Protection Against Mycobacterium tuberculosis Aerosol Challenge in a Preclinical Mouse Model

Introduction
Tuberculosis has led to the most deaths worldwide of any infectious disease over the last four years [1].

Mycobacterium tuberculosis (MtB) is becoming increasingly drug-resistant and BCG vaccine has limited effectiveness.

Results & Discussion

Lung Homogenization Does Not Inactivate D29
- Phage D29 titer after lung homogenization was 12.0 ± 0.03 log(PFU/mL).
- Control titer before homogenization was 12.2 ± 0.04 log(PFU/mL).
- No significant difference (p > 0.5; n=3 each).

MtB H37Rv Susceptible to Phage D29
- Control plate with MtB (no phage added) incubated for 3 weeks resulted in 38 CFU.
- Plate with MtB and phage D29 addition (2 replicates) resulted in 1 CFU or 2 CFU.
- Phage D29 efficiently lysed MtB H37Rv.

Dose Simulation Matches In Vivo Experiment
- Measured dose in vitro matched dose simulation for amount of phage nebulized.
- Phage active in lungs 90 minutes post-exposure, indicating challenging mice with MtB 30 minutes after phage exposure would be acceptable.
- Higher dose of phage in the lungs of mice than in any other study that used nose-only inhalation.

Materials & Methods
Phage D29
- TEM of Mycobacterium phage D29 [2], which was amplified on Mycobacterium smegmatis in a titer of 5.5x10⁹ PFUs/mL.

Prophylactic Respiratory Delivery of D29 Provides In Vivo Protection against M. tuberculosis
- Approximately 1 PFU/alveolus, or 7.6 log(PFU/mouse), of phage D29 measured to be delivered prior to all bacterial challenge experiments.

In vivo results show a prophylactic effect.
- Poison statistics [11] useful for estimating the probability, P, that an alveolus will contain a certain number of phages, λ, knowing the average number of PFU/alveolus, A.

Phage D29 appears promising for prophylactic protection against MtB, exhibiting capacity to significantly reduce bacterial levels in the lungs of mice.

Vibrating mesh nebulizer coupled with a nose-only exposure system is a good choice for delivering phage D29 in animal exposure experiments; delivered an average 7.6 log(PFU) to lungs of each of 12 mice in ~20 minutes.

Lysis of bacteria in the alveoli by phage is effective.

Development of cocktails active against tuberculosis is of interest; developed exposure system may be used to test efficacy of each anti-tuberculosis phage.

Lungs of nebulized mycobacteriophage cocktail aerosol to the lungs may be a valuable intervention to provide extra protection to health care professionals frequently exposed to infectious active cases of tuberculosis and to individuals in regions with high tuberculosis transmission rates.

Complete prophylaxis in humans may be achievable as higher doses could be more easily achieved; human clinical trials are of interest.

Conclusions

Female C57BL/6 mice age 6-6 weeks weighing 14-16 grams, have a minute ventilation of ~22 mL/min [4], the mice were trained to remain calm in the restraint tubes.

Nebulizer Bacteriophage D29 was delivered to mice within 30 minutes of phage D29 pre-treatment using a Wisconsin Madison aerosol chamber; low dose challenge delivered ~10² PFU/mouse and ultra-low dose challenge delivered ~10¹ PFU/mouse (image from [5]).

Further details regarding this poster is available in:


Acknowledgements
The authors thank Berrie Faulkner for manufacturing the first plunger. The vibrating mesh nebulizer was provided by Jim Fink and Ronan MacLoughlin (Aerogen Ltd.).

References
Further details regarding this poster is available in:

