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JOURNAL CLUB

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ARTICLE

"Host and pathogen response to bacteriophage engineered against *Mycobacterium abscessus* lung infection". Nick JA, et al. *Cell* 2022;185(11):1860-1874 e12. DOI: 10.1016/j.cell.2022.04.024. PubMed PMID: 35568033; https://pubmed.ncbi.nlm.nih.gov/35568033/

CLINICAL QUESTION

Mycobacterium abscessus pulmonary disease is associated with frequent treatment failure due to high intrinsic antibiotic resistance, and thus has been identified as one of the most attractive candidates for phage therapy. This study was the first report of successful treatment of *M. abscessus* pulmonary disease, and the most complete description to date of host and bacterial response to phage.

SUMMARY

Background: Bacteriophages (phages) are viruses that selectively infect bacteria and can replicate lytically, resulting in bacterial death. Lytic phages are increasingly being evaluated for therapeutic use in difficult-to-treat infections [1]. *M. abscessus* is a nontuberculous mycobacteria (NTM) with high levels of intrinsic and acquired antibiotic resistance that limit treatment options and contribute towards poor outcomes [2]. While *M. abscessus* has been cited as a potentially important target of phage therapy [3], and is among the most common infections referred for phage therapy [4], there are few reports of its use[5,6].

Persons with cystic fibrosis (pwCF) represent the most vulnerable population for NTM lung disease [7]. *M. abscessus* infections have been associated with a greater decline in lung function compared to typical CF airway pathogens [8], and when refractory to treatment impose a contraindication to lung transplant [9,10]. In advanced CF lung disease, antibiotic treatment of *M. abscessus* infection may be unsuccessful due to structural changes to the lung, in particular cavities and regions of dense consolidation and parenchymal collapse, which limit therapeutic concentrations of antimicrobials. *M. abscessus* is also known to form biofilms within the CF airway [11]. In microenvironments within the damaged lung, mycobacteria may survive for extended periods without oxygen or nutrients by shifting into a non-replicating "persister" state that may further thwart conventional antimicrobials and host defense [12]. It is possible that these same factors could thwart phage treatment, as well as co-infection by closely related lineages of *M. abscessus*, selection of a more phage-resistant isolate [3,4], or development of neutralizing antibodies against the phage[5,13]. In a broader disease context, if substantial killing of *M. abscessus* was achieved, the resulting niche could become occupied by other opportunistic pathogens. Experiences with phage therapy against *P. aeruginosa, S. aureus*, and *Burkholderia dolosa* in CF lung disease have demonstrated examples of improvement, but without clear evidence of eradication of the target infection [14]. In some cases, phage therapy resulted in the emergence of phage-resistant and antibiotic-resistant isolates, as well as new pathogens [14].

This paper describes the compassionate use of two engineered mycobacteriophages for a young pwCF and *M. abscessus* subspecies *abscessus* lung disease, whose infection was refractory against intensive multidrug antibiotic treatment. Extensive collection and biobanking of isolates and clinical samples throughout the duration of his infection pre- and post-phage therapy enabled an in-depth assessment of the effect of phage treatment on *M. abscessus* infection in the CF lung.

Methods and Results:

1. Clinical Summary From Time of Estimated Initial Infection Through Day 500 of Phage Treatment (Figure 1)*:* Clinical parameters plotted versus time in days, with initial phage administration labeled as Day 0. *M. abscessus* airway cultures turned predominantly negative after approximately

3 months of phage therapy, and *M. abscessus* was not cultured from the explanted lungs at the time of transplant.

2. Selection of phages, and monitoring for resistance (Figure 2): *M. abscessus* isolates recovered throughout the course of his infection were tested against a panel of phages. There was no evidence of resistance by the bacteria against the phage.
3. Radiologic Changes During Phage Therapy (Figure 3): CT evidence of advanced CF lung disease and *M. abscessus* infection were evident prior to phage therapy, with areas of improvement seen by day 81, and greater improvement by day 229 of phage.
4. Biomarkers of *M. abscessus* killing (Figure 4): Airway cultures were combined with sputum qPCR, urine LAM and serum anti-*M. abscessus* antibodies to accurately determine the course of phage therapy.
5. Response of *M. abscessus* to phage (Figure 5). Whole genome sequencing of *M. abscessus* isolates (n=40) recovered over the course of the infection and treatment demonstrated a reduction in genetic diversity over time, and the isolates became less antibiotic resistant with phage treatment.
6. Antiphage-neutralizing antibody response to phage (Figure 6). There was a late increase in neutralizing antibodies against one, but not both phages.

Conclusions:

- Phage combined with antibiotic treatment resulted in apparent eradication of *M. abscessus.*
- There was no evidence of resistance or increased *M. abscessus* virulence in response to phage therapy.
- Culture-independent markers correlated well with standard airway cultures, and strongly supported the conclusion of significant decrease in *M. abscessus* burden.
- In this subject, antiphage-neutralizing antibodies titers developed gradually against one phage, indicating different within-subject immune response to specific phages.
- Control of *M. abscessus* allowed for a successful lung transplant, without evidence of post-transplant infection.

Remaining questions:

- To what extent is this case representative of future cases of successful phage therapy against *M. abscessus* pulmonary disease?
- What contribution did ongoing antibiotics and the introduction of the CFTR modulator elexacaftor/tezacaftor/ivacaftor (E/T/I) have toward treatment success?
- When is the optimal time for phage treatment of *M. abscessus* lung disease, given the risk of neutralizing antibody development?
- Can the frequency and duration of phage therapy be reduced once a response has been detected?
- Why does phage therapy sometimes fail?

GROUP OPINION

Treatment of antibiotic-refractory *M. abscessus* by bacteriophage is in its infancy. While this and several other cases have succeeded, a number of others have failed to demonstrate clinical and or microbiologic improvement[15]. Results from the analysis of *M. abscessus* in this case and others support the conclusion that the relative genetic stability of the pathogen favors phage treatment, with a decrease in genetic diversity and no sign of antibiotic resistance or new pathogen acquisition. The role of the neutralizing

antibody response remains unclear and a potential cause of treatment failure, with wide variations seen between subjects and a poor correlation with treatment response[15]. A more systematic analysis of phage response against *M. abscessus* is clearly needed, and this report helps to provide a framework for designing such a trial.

Summary Statement: In the setting of compassionate use, two engineered mycobacteriophages were administered intravenously to a male with treatment-refractory *Mycobacterium abscessus* pulmonary infection and severe cystic fibrosis lung disease. The subject received lung transplantation on day 379 of phage and antibiotic treatment, and systematic culturing of the explanted lung did not detect *M. abscessus*. The report describes the course and associated markers of successful phage treatment of *M. abscessus* in advanced lung disease.

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