The Wonderful World of Mycobacteriophages: Discovery, dynamics, collusion, and therapy

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LeHigh University
October 11th, 2019
Two very sick patients

9\textsuperscript{th} October 2017

- Email message from Dr. James Soothill in London:
- Two patients with difficult to treat disseminated *Mycobacterium abscessus* infections:
- Cystic Fibrosis; Double lung transplant

2\textsuperscript{nd} November 2017

- Strains received in Pittsburgh; subcultured

Both strains highly antibiotic resistant
Patients are at high risk
Could phages be used therapeutically?
CT-PET: Pre-treatment

A

Pre-treatment

1

2

tigecycline
imipenem
amikacin
moxifloxacin
clarithromycin
linezolid
linezolid
amphotericin
clofazimine
Bedaquiline
Rituximab
clofazimine
Bedaquiline
Rituximab
tacrolimus
tacrolimus
mycophenolate mofetil
methylprednisolone
Sternal wound and skin nodules

Pre-treatment
What are bacteriophages?

- Bacteriophages (phages) are viruses that infect bacteria
- Replication is host-dependent
- They contain their own genetic material
- Phages are host-specific; sometimes at strain level
- Phages are small (need an electron microscope to see)
- Many different types; mostly dsDNA tailed phages
- Maybe lytic or temperate
- Population is vast, dynamic, old, and highly diverse
Bacteriophages: The numerical majority

The phage population is vast
- $10^6 - 10^7$ phage particles/ml
- $10^{31}$ phage particles in biosphere

The phage population is dynamic
- 5:1 - 10:1 phage:bacteria
- $10^{23}$ phage infections per second

The phage population is old
- Capsid structural conservation suggests ancient origins
The Freezer!
Bacterial hosts for phage isolation: Targeting Actinobacteria
SEA-PHAGES: Phage discovery and genomics
A platform for integrating research and education

- Concrete beginnings offer equal opportunity of engagement
- Complex abstract processes contextualized for constructed learning
- Parallel projects facilitate peer-mentoring and development of flourishing scientist/educators

HHMI PHIRE program 2002-2019 (Pittsburgh); K-RITH/UKZN
HHMI SEA-PHAGES, 2008-2019 150 schools, >5,000 students (2019)
SEA-PHAGES vs Traditional Lab

COURSE TYPE COMPARISON

Mean Student Response

- Project Ownership Content
- Project Ownership Emotion
- Self-Efficacy
- Science Identity
- Science Community Values
- Networking

*** indicates statistical significance.
Growth in the number of sequenced Actinobacteriophages
Geographical distribution of sequenced actinobacteriophages
Limited diversity of mycobacteriophage morphologies
Welcome to the Actinobactériophage Database at PhagesDB.org, an interactive site that collects and shares information related to the discovery, characterization, and genomics of phages that infect bacterial hosts within the phylum Actinobacteria.

Phagehunting In South Africa
Teaching Assistants preparing for the 2013 K-RITH Mycobacterial Genetics Course at University of KwaZulu Natal, Durban, South Africa.

Gene Content Comparisons Now Available on PhagesDB
Dan Russell | Jan 23, 2018

Live Phage Stats

- **Actinoplanes** 1
- **Arthrobacter** 270
- **Brevibacterium** 2
- **Corynebacterium** 21
- **Gordonia** 382
- **Microbacterium** 229
- **Mycobacterium** 1800
- **Propionibacterium** 55
- **Rhodococcus** 55
- **Rothia** 1
- **Streptomyces** 222
- **Tetrasphaera** 1
- **Tusukumira** 2

- **Total Phages** 15575
- Found in 2019 520
- Found in 2018 2472
- Found in 2017 2817

- **Finished Genomes** 3041
- Available Fasta Files 3041
- Phamerated 3031
- # Phams 30382

- **In GenBank** 2716
Mapping the diversity of Actinobacteriophages

Definitions:

- **Cluster**: A group of phages with substantial nucleotide sequence similarity and shared gene content; e.g. Cluster A
- **Subcluster**: Subdivision within a cluster (by distinct ANI and other parameters) e.g. Subcluster A2
- **Singleton**: One of a kind phage with no close relatives

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Fantastic diversity of bacteriophages

A Network phylogeny

Pope et al., '15
Mosaic construction of phage genomes

- Phrann (N)
- Corndog (O)
- Brujita (I1)
- Squirty (F3)
- Gaia (X)
More than 50% of Actinobacteriophages are temperate

Lysogeny
(parasitism: no viral reproduction, no cell death)

Induction
(spontaneous/UV)

Lytic growth
(viral production/cell lysis)

Lytic (virulent) phages only do lytic growth
Temperate phages choose between lytic growth and lysogeny

Prophage
(integrated)

Turbid plaque

Temperate

Lytic

Clear plaque
Evidence of collusion: Collaboration between phages and bacteria to defend against phage attack

- Bacteria have numerous systems to protect against viral attack
- Temperate phages contribute many additional defense systems
- Defense is typically heterotypic (against a phage different to prophage)
Discovering prophage-mediated defense systems

Because these can be highly specific, need:

- A large collection of phages known to infect a common bacterial strain
- A subset of temperate phages forming stable lysogens in that strain
- Genomic definition of the phages
- Expansive diversity of the phages that spans completely distinct genome sequences, and variants.
Prophage-mediated defenses are often highly specific.
The search for bacteriophages for GD01 & GD02

Goals:

- Identify three or more phages that infect *M. abscessus* GD01 and GD02
- Phages should be genomically distinct to minimize cross-resistance to the phages
- Phages should be lytic, and not temperate
- Phages should be grown to high-titer and stable
Strategies for identifying of *M. abscessus* phages

Three main approaches:

1. Screen individual *M. smegmatis* phages in the archive for that infect *M. abscessus* GD01/GD02
2. Enrichment of large pools of extant phages
3. De novo phage isolation

**Bad news:** One patient died ~6 weeks after starting phage search

**Good news:** Within ~2 months identified one good phage and engineered/genetics two other phages for other patient
Narrowing the search: Genome type correlates with host range

• A subset of phages isolated on *M. smegmatis* also infect other mycobacterial species

• ~10% of all *M. smegmatis* phages also infect *M. tuberculosis* H37Rv, but constrained to genomic types:
  • Clusters A2, A3, G, K, AB are best candidates for broader host ranges (spans total ~200 isolates)
  • Correlation is imperfect, and not all phages of a cluster/subcluster have identical host ranges

De novo isolation identified few new phages for *M. ab GD01*, and no new genomic types
Few *M. smegmatis* phages infect *M. abscessus* GD01

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^1Phage lysates were grown on *M. smegmatis* mc^155, except lisa and DS6A, grown on GD01 and M. tb respectively.
^2Plaque forming units ml^-1
^3Phages tested in an initial screen from which phages for a therapeutic cocktail were selected.
^4Additional phages tested after the initial therapeutic phages were selected.
Screening the extant collection of *M. smegmatis* phages: Phage Muddy infects and kills *M. abscessus* GD01

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Few *M. smegmatis* phages efficiently infect *M. abscessus* Muddy efficiently infects *M. abscessus* GD01 M. ab GD01 and M. ab GD02 differ in their susceptibilities
Phage #2: Engineering a lytic derivative, ZoeJΔ45

B. M. smegmatis mc²155

C. M. abscessus GD01

Kathleen Cornerly
Rebekah Dedrick
Phage #3: Mutational Expansion of host range of a lytic derivative of phage BPs

- BPs & BPsΔ33 plate inefficiently on *M. abscessus* GD01
- Plaques picked from *M. abscessus* have expanded host range
- Mutants grown on *M. smegmatis* infect *M. abscessus* GD01

BPs HRM’s have mutations in the phage portal gene (gene 3)
The three musketeers
Phage killing of *M. abscessus* GD01

Bacteria & phages were incubated in liquid culture for 96 hours, then plated for survivors on solid medium.

Good killing by each individual phage with some variation. Complete killing and no survivors with cocktail.
The first phage therapy of a mycobacterial infection

- A three-phage cocktail against *M. abscessus* GD01: Muddy, ZoeJΔ45, BPΔ33HRM
- Phages were grown to high titer; purified through two rounds of CsCl equilibrium density centrifugation
- Stocks at $10^{12} - 10^{14}$ pfu/ml. Cocktail at $10^{11}$ pfu/ml; stable.
- Dialyzed extensively into PBS; demonstrated to have negligible CsCl remaining; negative for LPS toxicity.
- Multi-stage approval process for importing and emergency compassionate use of unlicensed drugs in the UK
- Approved as non-GMO
- Administered IV 2x-daily; $10^9$ pfu/dose; Topical application to sternal wound and skin nodules
- Monitor clinical response; phage in sputum/serum; immune response; phage resistance
Summary of outcomes

- No adverse reactions
- Phage recovered from some patient samples, consistent with *in vivo* replication
- Improved lung function
- Weight gain
- Infected liver node resolved by 6-weeks
- Closure of the sternal wound
- Skin nodule resolution (incomplete)
- No neutralizing antibodies detected
- Phage resistance not observed in any subsequent isolate
- Patient able to return to normal routine
Recovery of phage from patient samples
CT-PET: Pre- and post-treatment

A

Pre-treatment

Post-treatment

B

Pre-treatment

Post-treatment
Resolution of wounds and skin nodules
Helen, GH, IC, Mum
Not one cure for them all

- Received about 40 NTM clinical isolates (mostly M. abscessus)
- Screened with ~20 ‘best’ phages
- ~40% of strains not susceptible to any of the phages
- Of other ~60%, many (~40%) only infected by one phage
Opportunities and challenges for phage treatment of NTM infections

Opportunities
- Personalized cocktails of matched phages may be effective for treating M. ab and other NTM infections
- IV administration is safe
- Resistance not observed (yet)
- Possibility of synergies with antibiotics
- Have started therapy on three addition patients.

Challenges
- Only ~60% of M. ab clinical isolates are sensitive to at least one phage; similar expected to MAC
- Screening is relatively time-consuming, long, tedious
- Engineering/genetics may be required
- Resistance profiles and mechanisms are unknown
- Monotherapy possibilities are unclear
- Response in immune-competent patient is unclear
- Aerosol administration may be possible
Summary

• SEA-PHAGES promotes student engagement and phage discovery
• Considerable potential for phage therapy of NTM infections
• Personalized treatments in progress
• Cocktail for general treatment of *M. abscessus* infections not currently possible
• Large collection of phages fuels therapeutic advancement
• Potential for therapy of TB considerable
• Need to understand phage dynamics *in vivo*
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Summary

SEA-PHAGES is an inclusive Research Education Community

Integration of research and educational missions promotes sustainable success in both

Bacteriophage discovery and genomics is a well-suited platform for student engagement

The number and diversity of phages promotes project ownership and an endless supply of novelty

SEA-PHAGES addresses basic questions in phage population but fuels translations including therapeutic interventions
TB phage therapy: challenges and opportunities

- Potential for TB combination treatment with antibiotics to a) reduce treatment length, b) reduce antibiotic resistance
- Potential for controlling MDR-TB and XDR-TB infections
- Need phages that infect broad spectrum of clinical isolates
- Phages must be lytic; engineering is relatively simple
- Orthogonal phage resistance profiles
- LPS contamination is not a safety issue

- M. tb is genetically more homogenous than other pathogens (core vs pan-genome: ~93%)
- M. tb strains do not carry prophages
- M. tb drug resistance derives from single resistance combinations; not MDR transporters
- Phage-resistance may be associated with reduced fitness
- IV versus inhaled delivery; little known about access in vivo
- Clinical trials (I/II) should be low risk, phages have been identified, GMP needed, immune-compromised vs -competent