• Background on phage biology
• What is and how does phage therapy work?
• Phage pharmacology
Phage Biology

Phages are viruses that;

- Specifically infect bacteria
- Often result in the lysis of bacterial cells within minutes of infection
- Co-evolve with bacteria
- Are numerous and environmentally ubiquitous
Phages exist in unlimited supplies and are easy to isolate and characterize.
Phage Biology

Phages are viruses that:

- Specifically infect bacteria
- Often result in the lysis of bacterial cells within minutes of infection
- Co-evolve with bacteria
- Are numerous and environmentally ubiquitous
- Highly diverse
**Inoviridae, ssDNA**

**Leviviridae, ssRNA**

**Fuselloviridae, dsDNA**
Tailed phages with dsDNA are the most common group (~90%) of phages infecting gammaproteobacteria
Phage Therapy
Scientists create drug to replace antibiotics

Breakthrough in fight against superbugs

Revolutionary New Antibiotic Alternative Could Save the World From Superbug 'Apocalypse'

BY AMELIA SMITH 11/6/14 AT 5:39 PM

Phages may be key in bacteria battle

By Damien McGuinness and Deborah Cohen
Health Check, BBC World Service

16 March 2013  Health

SILENT KILLERS: FANTASTIC PHAGES?

Bacteria Eaters

After breaking his foot five years ago, Toronto bass player Alfred Gertler got an infection that antibiotics couldn't cure. Doctors told him he might have to have his foot amputated.
Phage therapy is the treatment of bacterial infections with:

- **Virulent phages**
  - Direct infection with natural virulent (obligate lytic) phages

- **Phage derived compounds**
  - Holins
  - Lysins
  - Antibacterial proteins
  - Identification of small molecules that mimic phage proteins

- **Phages as vectors (delivering genes)**
  - Adding genes for bacteriocins
  - Interfering with antibiotic resistance genes
  - Inhibition of bacterial metabolism
  - Genes for wider host spectrum
Phage therapy has a number of advantages;

• **Specificity**
  — Possible to select activity against a single clone
  — Does not impact the normal bacterial flora
• **Phages increase in the presence of a suitable host**
• **Phages decay in the absence of a host**
• **“Environmentally friendly”**
However, there are also a number of disadvantages:

- Intracellular infections cannot be treated
  - Possibility to target alternate stages
- Risk for septic shock following treatment
  - Release of bacterial debris following cell lysis
- Circulating phages can be cleared
- Resistance
  - Bacterial resistance can be combatted by combining 2 or more phages (cocktails)
- Uncharacterised phage genes
Does it work?

• Phages are the main regulators of bacterial densities in nature
• Routinely used in a number of centres in Eastern Europe
  — Eliava Institute (Tbilisi, Georgia)
  — Institute of Immunology and Experimental Therapy (Wroclaw, Poland)
• Approved for use in the US and Europe in some food applications
  — GRAS approvals from FDA (Listex P100, Micreos Food Safety; ListSheild, Intralytix
  — EFSA approval (Listex, Micreos)
• Phage therapy is a special case of host – parasite coevolution of phages and bacteria
  — ESS of phage and bacteria interactions
  — Phage pharmacology
  — Phage therapy strategies
ESS of phage -bacteria interactions

Phages and bacteria are confined to two Evolutionary Stable Strategies (ESSs)

Evolution of host resistance and phage virulence

“Arms race”

Fitness (resistance / virulence)

Evolution of host tolerance, phage latency time and lysogenic conversion genes

“Mutualism”
Phage Pharmacology
Pharmacology: Pharmacodynamics

What is the mass of 1 mole of phages?

Phage T7: 49 metric tonnes

1 mole of penicillin V

= 350 grams

10 day course (20g)

= 0.06 moles

In practice approximately only \(10^{11}\) phages can be applied = \(10^{-13}\) moles.

→ It is essential that the treatment results in a productive infection
Pharmacology: Pharmacodynamics

• Studies have addressed phage therapy in general or as an alternative to antibiotic treatment

• Levin and Bull (1996) mathematically modelled mice infection experiments carried out by Smith and Huggins (1982)

• Simulation of phage and bacteria population parameters
  – Density of phage-sensitive bacteria
  – Density of phage-resistant bacteria
  – Intensity of mouse’s immune response
  – Density of the phage

• Comparison with chemostat experiments
Pharmacology: Pharmacodynamics

S = Sensitive bacteria
R = Phage resistant bacteria
I = Immune response.

At the threshold, the concentration of bacteria is too high and the host dies.

Levin BR & Bull JJ
Nature Reviews Microbiology 2004:2 166-173
Pharmacology: Pharmacodynamics

S = Sensitive bacteria
R = Phage resistant bacteria
P = Phages
I = Immune response.

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Pharmacology: Pharmacodynamics

- In experimental systems complete clearance is almost never observed
  - Discrete variables that are not accounted for in the models
- Interference
  - Interaction with bacterial debris and other components
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- Interference
  - Interaction with bacterial debris
  - Competition between phages
Pharmacology: Pharmacodynamics

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- Interference
  - Interaction with bacterial debris
  - Competition between phages
Pharmacology: Pharmacokinetics

- Phage Immunogenicity
  - Pre-existing antibodies
  - Not all phages are created equal

- Immune Clearance
  - Limits the number of phages able to reach an infection site
Pharmacology: Formulation and Delivery

- Different delivery mechanisms could increase the numbers of phages reaching an infection
  - Focus has primarily been topical application
    - Simple liquid phage culture
    - Research into aerosolisation and hydrogel delivery

- Increasing Activity
  - Synergy with other compounds e.g. antibiotics
  - Phage derived depolymerases
  - Phage selection

- Reducing clearance and increasing stability
  - Encapsulation
  - Lyophilisation
General Conclusions

Virulent Phages;

• Are cheap and relatively easy to isolate
  – Reduces the risk of resistance developing

• Are highly specific
  – Target single or small number of bacterial strains

• Can be fast acting
  – Phages are selected on the basis of virulence characteristics e.g latency period, burst size

• Are large and diffuse poorly

• Cannot be given in large doses
  – **Essential** to establish a productive infection
General Conclusions

Phage therapy;

• **Is not a “magic bullet”**

• **Works in theory IF phage/bacterial co-evolution AND pharmacology are considered**
  – Some issues can be addressed through pharmacology research

• **Requires research into more complex scenarios**
  – Multi-phage/multi-target systems
  – Complex formulations

• **May require alternative drug licencing pathways**
  – Difficulties recruiting for clinical trials
  – Clinical testing often not economically viable

• **Is already in limited clinical use**
  – Also approved for use in some food applications
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