The phageome; a key element of the microbiome and a source of novel therapeutics

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Celebrating >100 years of phage research

Narrow spectrum and targeted antimicrobials - Phage therapy in a post-antibiotic era.

The human microbiome is dominated by bacteriophages – role in shaping the microbiome.
Phage Therapy in the spotlight

Eliava Institute in Tbilisi, Georgia (Est 1923) – bacteriophage preparations & tailor made personalised therapies

How Phage Therapy Is Saving Lives

Phage Biotechnology Companies

Fish et al., 2016 Journal of Wound Care
Searching the Microbiome for Phage Therapy

- S. pneumoniae & P. aeruginosa phages
- S. mutans phage
- E. coli phages

Bacteriophages φMR299-2 and φNH-4 Can Eliminate Pseudomonas aeruginosa in the Murine Lung and on Cystic Fibrosis Lung Airway Cells

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The human gut virome, also known as the gut phageome, refers to the whole community of viruses in the gut, most of which are bacteriophages.
Human gut virome/phageome: Some facts and figures

- Estimated there up to 4 times more phages than bacteria
- Your body absorbs about 30 billion phages per day
- Metagenomic sequencing: ~6% of reads have bacteriophage origin.
- 35-2,800 phage types per sample.
- For ~90% no host can be predicted and in some cases up to 80% cannot be assigned to any known morphological family.

7 x 10^9 people on earth

10^9 – 10^{10} VLPs/g in human faeces

Hoyles et al., Res. Microbiol. 2014
Phages – an important element of the microbiome

- Acne
- Antibiotic-associated diarrhoea
- Arthritis
- Asthma/allergies
- Atherosclerosis
- Autism
- Autoimmune diseases
- Cancer
- Dental caries
- Depression and anxiety
- Diabetes
- Eczema
- Gastric ulcers
- Infections
- Inflammatory bowel diseases
- Malnutrition
- Metabolic syndrome
- Necrotizing enterocolitis
- Obesity
- Parkinson’s disease
- Psoriasis
- Vaginosis
Strain level phage data gives high resolution picture of the microbiome.

Phage metagenomics provides a different view of the microbiome.

Phages are non-living, so more robust than bacteria.

Phage can act as reporters of what bacteria “were present”.

Phage can act as biomarkers in health and disease.

Gut phages as therapeutic agents e.g. FMT.

Signal of what is further up GIT.

Phage as mediators of the microbiome.
- Robust to improper storage and freeze-thaw cycles
Bacteriophage as biomarkers/diagnostics

Viromes of one year old infants reveal the impact of birth mode on microbiome diversity

Angela McCann, Feargal J. Ryan, Stephen R. Stockdale, Marion Dalmasso, Tony Blake, C. Anthony Ryan, Catherine Stanton, Susan Mills, Paul R. Ross, and Colin Hill

- Bacterial OTUs could not distinguish a difference in the microbiome of these infants
- Results indicate that vertical transmission of viral communities from mother to child may play a role in shaping the early life microbiome.
Bacteriophage adhering to mucus provide a non–host-derived immunity

1) Epithelial cells secrete mucus.

2) Mucus is shed with mucus.

3) Adherent phage form anti-microbial layer.

4) Mucus-adherent phage have increased chance of replicative success.

5) Phage and bacteria are shed with mucus.
• Flourishes when microbiome is disrupted by antibiotics
• Relapses are common – up to 35%
• Often has a lethal outcome - a 1-year mortality rate of nearly 16% was observed among rCDI patients (Kuntz et al., 2017)

FMT has 80-90% success rate in rCDI disease resolution.
Faecal Transplantation

Study of viral transfer following FMT from 3 donors to 14 rCDI patients – 1 year

Long-term colonisation with donor bacteriophages following successful faecal microbiotal transplantation

Microbiome

APC Microbiome Ireland
Intersecting Food & Medicine
Individual’s viromes are highly unique

Virome gives unique view of the microbiome

Strain level resolution - metagenomics
Relative abundance of transferred donor contigs is highly variable across donor-recipient pairs.

Different viral colonization patterns are observed.

**Conclusion**
- FMT is a successful treatment for rCDI
- Given that >90% of the phageome have unknown function, more research is required for FMT in non-life threatening conditions
Sterile Faecal filtrate transfer

Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With Clostridium difficile Infection

Stephan J. Ott,1,* Georg H. Waetzig,2,* Ateequr Rehman,3,* Jacqueline Moltzau-Anderson,4,3,4 Richa Bharli,5 Juris A. Grasis,5 Liam Cassidy,5 Andreas Tholey,6 Helmut Fickenscher,7 Dirk Seegert,2, Philip Rosenstiel,2,5 and Stefan Schreiber3,2,6

RESULTS: In all 5 patients, FFT restored normal stool habits and eliminated symptoms of CDI for a minimum period of 6 months.

A key advantage of FFT is the avoidance of all risks inherent to the transfer of living microorganisms. Further advantages include the potential for standardization and for the development of a robust, inexpensive, and patient-friendly formulation (ie, capsules filled with freeze-dried FFT preparations without the need to conserve living bacteria or spores). When contemplating the intersection of successful therapies related to FMT (classic FMT, spores, and FFT), it appears plausible that the active agent(s) of any FMT therapy are not living bacteria, but rather bacterial components, antimicrobial compounds of bacterial origin (eg, bacteriocins), or bacteriophages contributing to the normal intestinal microenvironment. These could be common to all successful FMT therapies and even rather unspecific regarding the bacterial strain(s) used for therapies.
Faecal Viral Transplantation in a Mouse Model

1) Distribution of the microbiome using an antibiotic cocktail of penicillin and streptomycin
2) Administer an Faecal Viral Transplant (FVT)/inactive FVT
3) Collect faecal samples
4) Examine effect on bacteriome and virome via NGS

FVT sample
Centrifugation and filtration removes all microbes except phages

Autochthonous Donation

16 mice acclimatised
Days 1-5
16 mice receive antibiotic treatment
Days 5-7

Donor faecal sample

n=8

Group 2 receives heat treated phage control

Secondary Faecal Transplantation

Day 12

T1
T2
T3

Donor FVT sample

Centrifugation and filtration removes all microbes except phages

FVT sample

Heat & nuclease treatment

16S rRNA Sequencing
Shotgun Sequencing

Illumina MiSeq NGS

Bacteriome and Virome DNAtraction from samples Collected

Metabolites
Bacterial Components
Viruses & Phages

Bacteriome
Virome
Ellipses represent 70% confidence intervals.

- **PCoA plot** of unweighted UniFrac distances supports the separation of the **bacteriome** of mice into significantly different clusters over time depending on the content of the gavage they received, whether it be heat treated or viable phages via FVT.

- Administration of FVT appears to **accelerate recovery** of FVT mice to the pre-treatment state more rapidly than control mice.
Conclusions

- **Phageome as a source of phage therapy solutions** - Saviours in a post-antibiotic era.
- Given the importance the microbiome in human health, the **phageome is a key factor** that needs research.
- Phage metagenomics as a **robust and reproducible** tool in microbiome research.
- Phage component provides another (essential?) dimension in human microbiome research.
- Phageome: a new source of **biomarkers** for human health or disease?
- Viral transplantation following FMT leads to **long term colonization of phages** in a donor-recipient specific manner. Phages involved in disease resolution?
- Preliminary research into **FVT in animals and humans** suggests a role for the phageome in **reshaping the microbiome** and in **disease resolution**.
Thank you
&
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