Phage-mediated immunity or Snot and viruses

"ape2phage" by Mark Hatay

Forest Rohwer
San Diego State University
Viruses infect Bacteria, Archaea and Eukarya

Phage are viruses that infect Bacteria

Virus-like particles (VLPs) are what we count under the microscope

Lytic versus lysogenic behavior

Proviruses & induction

Super-infection exclusion
Cellular life exists so that viruses have something to eat

There are $\sim 10^{31}$ viruses in the world

At least 100 million viral species on Earth

Major predators on the planet
- determine which strains survive (Kill-the-Winner; Red Queen)
- control number of microbes (top-down)

Most of genetic diversity on the planet is viral
- move DNA around the planet
- important genes are evolving in viruses

The vast majority of viruses are uncultured...study them using metagenomics (Breitbart et al. 2002)

The main differences between holobionts are the viruses
Phecal phage in monozygotic twins

Four sets of monozygotic twins and their mother, sampled three times over one year, yielded fecal samples. These samples were used to isolate viral-like particles and subjected to shotgun sequencing.

Co-twins and their mothers have a significantly greater degree of similarity in their fecal bacterial species composition than do unrelated individuals.
Most of the human fecal virome is unknown

>70% unknown in viromes ($t$BLASTx vs NR) versus <10% unknown in microbiomes (which are viruses...)

fecal viromes & microbiomes

$\downarrow$ BLASTx

against COG database

considered significant if E value $<10^{-5}$

COG
Most of the metabolic variation in the human gut is encoded by viruses.

**Fecal viromes & microbiomes**

- **BLASTx** against COG database
- Considered significant if E value < $10^{-5}$
- Relative % of different metabolic functions

**Phage are cool**

**Bacteria are boring**
**Intrapersonal diversity** is very low over time. >95% of virotypes retained over 1 year (Maxiphi).

- Fecal viromes
- CD-Hit to cluster
- Hellinger distances to determine similarity amongst viromes
- GAAS/PHACCS/Maxiphi analysis yield similar results

**Interpersonal diversity** is high.

Viromes are the variation between you & the person next to you.
Mucus associated phage
Hired killers of the immune system?

Mucus forms the initial barrier between 'us' and the environment.

Lungs, sinus, oral cavity, gastrointestinal track.

Mucus forms entire living surface of many organisms.
What is in mucus?

- salts
- sugars
- lipids
- proteins
- huge array of macromolecules

Mucins are large glycoproteins, whose entanglement forms the gel-like coating termed ‘mucus’

also phage & microbes...
Quantify phage concentration in mucus compared to surrounding environment

suck off mucus & take an surrounding environmental sample
quantified bacteria & phage using epifluorescent microscopy
phage-to-bacterium ratio (PBR)
Phage are enriched relative to microbes on mucosal surfaces

most environments have a 10 phage per 1 microbial cell

mucus has almost 40 phage for every microbial cell

change in phage-bacteria encounter rates (i.e., mass action)
Phage bind to mucus & specifically mucins

TC cells w/ & w/o mucus

T4 phage washed across surface

layered with *E. coli* host in top-agar

count plaques

agar plates w/ mucin, DNA or protein (BSA)
Phage kill bacteria when attached to mucus (i.e., not inactivated)

TC cells w/ & w/o mucus
↓
incubate w/ T4
↓
washed & layered for *E. coli* for 4 hrs
↓
phage and bacteria quantified via epifluorescence
Phage protect TC cells from bacteria

TC cells w/ & w/o mucus
↓
incubate w/ phage T4
↓
washed & layered E. coli overnight
↓
TC cells were stained with live/dead and analyzed via flow cytometry
Bacteriophage Adherence to Mucus (BAM) - acquired immunity -

1) Epithelial cells secrete mucus

2) Phage adhere to mucus

3) Aderent phage form anti-microbial layer

? specific or non-specific ?
Ig-like domains on phage

T4-like coliphage encode three Ig-like domains displayed via the highly immunogenic outer capsid protein

155 copies of Hoc protruding from capsid surface

these domains are used in phage display systems

Hypothesis: Phage adhere to mucins through capsid displayed Ig-like domains (e.g., Hoc)
A little more about the Ig-like & lectin decoration proteins on phage

Like antibodies and T-cell receptors, these proteins can accommodate massive sequence variation (>10^{13} potential alternatives)

Hypervariability is conferred by targeted mutagenesis through a reverse transcription mechanism

There are also hypervariable C-type lectin decorations

Decoration proteins are usually dispensable for phage growth in the laboratory

Minot et al. showed that phage in the human gut encode a large population of hypervariable decoration proteins (PNAS; 2012)
Diversity of Ig-like & lectin domains in viral metagenomes

- common in mucus-associated viromes -
Hoc Ig-like domain is necessary for phage to bind to purified mucin

agar plates w/ mucins, DNA or protein (BSA)

\[ \downarrow \]

T4 phage +/- Hoc

washed across plate surface

\[ \downarrow \]

layered with \textit{E. coli} host in top-agar

\[ \downarrow \]

count plaques

- Hoc knockouts of phage T3 behave the same -
Ig-like domain is necessary for phage to bind to purified mucin

Multiple Particle Tracking (MPT)

fluorescence labeled T4 and hoc-phage in 1% (w/v) mucin & buffer control

recorded trajectories over temporal resolution of 100 ms for 30 sec

calculated effective diffusivities ($<D_{eff}>$) of phage
Bacteriophage Adherence to Mucus (BAM) - acquired, adaptive & specific immunity -

1) Epithelial cells secrete mucus

2) Phage adhere to mucus through Ig-like domains

3) Adherent phage form anti-microbial layer

- Ig-like domains
- Glycan residues
- Mucin protein backbone
BAM mathematical model

encounter rate between phage and microbe...

\[ k_d B \Phi \]

diffusive rate constant is given by...

\[ k_d = 4 \pi RDN_A \]

probabilities of an encounter between a phage and microbe, both with and without the mucus layer...

\[
\frac{Q^{mu}}{Q^{aq}} = \frac{k_d^{mu} \cdot B^{mu} \cdot \Delta t^{mu,\text{diffusive}}}{k_d^{aq} \cdot B^{aq} \cdot \Delta t^{aq,\text{diffusive}}} \cdot \frac{\Delta t^{mu,\text{adhered}}}{\Delta t^{mu,\text{diffusive}}} 
\]
Mucus is good for phage & bad for microbes

A phage...

15 times more likely to find host!

A microbe...

14 times more likely to die!
Bacteriophage Adherence to Mucus (BAM) Immunity

- acquired & specific -
- works via hypervariable, Ig- & lectin-like domains -
- link between innate immune system & phage -

1) Epithelial cells secrete mucus

2) Phage adhere to mucus through Ig-like domains

3) Adherent phage form anti-microbial layer

4) Positive selection of mucus-adherent phage results in adaptation

5) Phage and bacteria are shed with mucus

? first anti-microbial immune system ?
Rohwer Lab - SDSU
Katie Barott
Jeremy Barr
Lance Boling
Jose Evangelista
Jeremy Frank
Mike Furlan
Juris Grasis
Allison Gregg
Eric Hester
Victoria Hosford
Benjamin Knowles

Nicole Hanson
Dana Willner (UQueensland)
Florent Angly (UQueensland)
Matt Haynes (now at JGI)

Edwards Lab - SDSU
Rob Edwards
Robert Schmieder

Mya Breitbart - USF

Doug Conrad - UCSD
Anca Segall - SDSU

Math Guys - SDSU
Peter Salamon
Ben Felts
Jim Nulton

Relman Lab - Stanford
David Relman
David Pride (UCSD)

Paul Rainey - Massey Uni

Gordon Lab - WU
Jeffery Gordon
Alejandro Reyes