MUCOSAL MICROBIOMES WITH HIV INFECTION AND PROTECTION

Scott A. Handley, PhD
Assistant Professor
Department of Pathology and Immunology
Washington University School of Medicine
Microbiomes in Health and Disease

Norman JM, Handley SA and Virgin HW. Gastroenterology. 2014. May;146(6) 1459-69
What is the Virome?

Virgin et al., 2014 Cell 157:142
The Healthy Adult Human Enteric Virome in an Industrialized Nation

Circovirus
Retrovirus
False-positive Double-stranded DNA viruses
Tailed Phage
Non Tailed Phage
Anellovirus
Plant Viruses

Norman, JN, Handley SA. et al.
The Healthy Adult Human Enteric Virome in a Developing Nation

Monaco et al. 2016 Cell Host & Microbe
HOW DO WE ANALYZE THE VIROME?
Virome Analysis Challenges

- No universal amplicon strategy
  - Lack of evolutionarily conserved target sequence (16S, 18S, ITS)
- High sequence divergence within viral groups
- Taxonomically poor sequence database
  - Lots of ocean viruses
- Short reads
  - Difficult for taxonomic assignment
    - High-sequence divergence
    - Large search space for false-positive classification
  - Difficult to assemble (chimeras, etc.)
    - Difficult to obtain genes for functional analysis
- Multiple genetic types
  - ds/ssRNA, ds/ssDNA
    - You can not just shotgun sequence stool and expect to sequence all types
Virome Characterization System

Read Based

Raw Sequence
  \[\xrightarrow{\text{bbtools}}\]
  High-quality Sequence
    \[\xrightarrow{\text{blastX}}\]
    Taxon assignment
      \[\xrightarrow{\text{R}}\]
      Richness - Diversity

Assembly Based

Assembled Contigs
  \[\xrightarrow{\text{megahit}}\]
  \[\xrightarrow{\text{bowtie}}\]
  An’vio
  \[\xrightarrow{\text{prodigal}}\]
  Binned Contigs
    \[\xrightarrow{\text{prodigal}}\]
    ORFs
      \[\xrightarrow{\text{interproscan}}\]
      Functions

  \[\xrightarrow{\text{genesomes}}\]
  Human Gastrointestinal Virome Database
This isn't a slide error. It is a major challenge in the field.

We really have no idea what the enteric virome looks like.
Virome Analysis Challenge: Viral Dark Matter
ENTERIC VIRUS ASSOCIATIONS WITH INFLAMMATORY BOWEL DISEASE (IBD)

Crohn’s Disease & Ulcerative Colitis
Environmental Component of IBD

Tailed-bacteriophage Expansion in IBD

United Kingdom

Chicago

Boston

Norman JN, Handley SA. Cell. 2015
Viral Contigs/Genomes Associated with Crohn’s Disease
Bacteriophage – Bacteria Relationship in IBD
BACTERIAL MICROBIOME AND VIROME IN AIDS

Simian Immunodeficiency Virus (SIV) in Macaques & Human Immunodeficiency Virus (HIV) in Humans
Expectation of “Dysbiosis”

Is There Enteric Bacterial “Dysbiosis” During AIDS?

- Low CD4+ T-cell counts (<200) does correlate with the emergence of bacterial enteropathogens

What About Specific Bacterial Taxa?

**SIV - Macaques**

- **CD4 Quartiles**
  - Top quartile
  - Bottom 3 quartiles
- **Log Fold Change**
  - Firmicutes
  - Bacteroidetes
  - Spirochaetes
  - Proteobacteria

**HIV - Uganda**

- **Log Fold Change**
  - CD4 > 200
  - CD4 < 200
- **Bacterial Taxa**
  - Actinobacteria
  - Bacteroidetes
  - Cyanobacteria
  - Firmicutes
  - Fusobacteria
  - Proteobacteria
  - Spirochaetes
  - Tenericutes

---

Enteric Viruses and GI Disease Pathology

- Uncontrolled SIV infection is associated with gastrointestinal disease
- GI disease is associated with leakage
- Uncontrolled SIV infection is associated with virome expansion
- Viral expansion is associated with GI pathology
SIV Vaccination Cohort (Barouch)

A)

No vaccine

SIV unchallenged

n=36

Vaccine

Sham

Ad alone

Ad + env

n=7

n=12

n=12

Intrarectal SIV challenge

Protected

n=8

Unprotected

n=21

Necropsy due to AIDS related illness

Survive

n=10

n=11
Figure 4. Gastrointestinal virus associations with the percentage of blood CD4 T cells and serum SIV load.

A) Circoviridae

B)...

C)...

Legend:
- Controller
- Protected
- Unprotected
- Detection of virus

Correlates of Protection

Enteric Viruses in Humans with HIV

VIROME CONSIDERATIONS: BENCH TO BEDSIDE
Phage alteration of bacterial communities

Phage interactions with host tissue/cells
Acknowledgements

Clinical Collaborators

IBD
- Miles Parkes (Cambridge)
- Ramnik Xavier (MGH)
- Dirk Gevers (MGH)
- Ali Keshvarzian (Rush)
- Ece Mutlu (Rush)
- Dermot McGovern (Cedars-Sinai)

AIDS
- Doug Kwon Lab (Ragon Institute)
- Dan Barouch Lab (Harvard University)

Oral Vaccines
- Vanessa Harris

Viral Infection and the Microbiome
- Larissa Thakray (Wash U)
- Mike Diamond (Wash U)

Washington University
- Skip Virgin (Vir)
- Thad Stappenbeck
- Jason Norman (Vedanta)
- Chandni Desai
- Barry Hykes
- Lindsay Droit