The Gut Microbiome and Inflammation in HIV-1 Disease

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Objectives

- Overview of HIV-induced mucosal pathogenesis and its clinical sequelae
- Introduction to gut microbiota and cross-study summary of microbiome alterations during HIV infection
- Wilson lab data on dysbiosis in untreated HIV-1 infection and links to inflammation
- Questions for group “digestion” and discussion
HIV-1 is a Mucosal Pathogen
Characteristics of HIV-associated Intestinal Mucosal Pathology

- High levels of viral replication in GALT during acute infection.
- Significant Th17 and Th22 infection and depletion
- Increased mucosal Treg : Th17 ratio
- Increased Mucosal DC and T cell activation
- Increased mucosal cytokine production
- Reduced intestinal epithelial cell (IEC) barrier resistance (leaky gut) and increased IEC apoptosis
- Migration of HIV-specific CD8 CTL into GALT that produce TNF-α and IFN-γ

*Intestinal homeostasis is restored slowly or incompletely on ART despite viral suppression in chronic infection*

Reviewed in Dandekar, Curr Opin HIV AIDS 2010
Consequences of Mucosal Pathology: Translocation of microbial products

- Levels of bacterial LPS were elevated in the plasma of HIV-1 infected subjects (Brenchley, Nature Medicine - 12, 1365 – 1371, 2006)

- LPS levels associated with blood T cell activation, an independent predictor of HIV-1 disease progression
Potential Clinical Consequences of Microbial Translocation

Deeks, Immunity 2013
Gut Microbiota

- Large, functionally stable community of bacteria
- ~100 trillion microbial cells
- ~1,000 bacterial species
- Unique to each individual
- Role in nutrient metabolism, barrier function, immunity
- Impacted by diet

HIV and the Microbiome: What we know….

- Compositional and metabolic differences (Dysbiosis) in gut bacteria were seen between HIV-infected versus uninfected controls (Ellis JAIDS, Perez-Santiago AIDS, McHardy Microbiome, Mutlu PLoS Path, Vujkovic-Cvijin Sci Trans Med, Lozupone Cell Host Microbe, Yu AIDS, Dillon MI, Vazquez-Castellanos MI)

- Some studies showed HIV-related changes in bacterial diversity

- Differences were seen in microbial alterations between mucosa and stool compartments

- The normal dietary associations with microbiota were disrupted during HIV infection in one study (Dillon)

- A “normal” microbiome was not restored in all subjects on ART (and ART may induce microbiome changes)

- Dysbiosis was linked to markers of immune activation and inflammation
RESEARCH GOAL: To determine whether changes in the gut microbiome are linked to features of HIV-1 pathogenesis.

In vitro modeling: Bacteria, HIV and mucosal immune cell interactions.
CHANGES IN THE GUT MICROBIOME DURING UNTREATED HIV INFECTION WOULD BE ASSOCIATED WITH COLONIC MUCOSAL INFLAMMATION AND CD4 T CELL DEPLETION

*Based on in vitro data from:
Dillon et al., JI 2010; Dillon et al., JI 2012; Steele, Retrovirology 2014
Clinical Study Design:
Characterization of Intestinal Dendritic Cells and Gut Bacterial Diversity in Untreated HIV-1 Infection

Enrolled: 24 HIV-infected and 14 seronegative control subjects

*Data published in Dillon SM, Mucosal Immunology, 2014.
### Microbiome SubStudy: Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Uninfected subjects</th>
<th>HIV-infected subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of subjects</strong></td>
<td>14</td>
<td>18</td>
<td>n/s</td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>31 (23-54)</td>
<td>32.5 (22-58)</td>
<td>n/s</td>
</tr>
<tr>
<td><strong>Male/Female Ratio</strong></td>
<td>9/5</td>
<td>13/5</td>
<td>n/s</td>
</tr>
<tr>
<td><strong>CD4 count (cells/ml)</strong></td>
<td>724 (468-1071)</td>
<td>425 (238-782)</td>
<td>P=0.0002</td>
</tr>
<tr>
<td><strong>Plasma Viral Load (HIV-1 RNA copies/ml)</strong></td>
<td>-</td>
<td>51350 (2880 – 207000)</td>
<td></td>
</tr>
<tr>
<td><strong>Years since first HIV-1 seropositive test</strong></td>
<td>-</td>
<td>4.75 (0.25-15)</td>
<td></td>
</tr>
<tr>
<td><strong>Body Mass Index (kg/m²)</strong></td>
<td>25.3 (18.5-32.3)#</td>
<td>25.4 (17.4-34.7)</td>
<td>n/s</td>
</tr>
<tr>
<td><strong>Ethnicity:</strong></td>
<td>n/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>11 (78.6%)</td>
<td>17 (94.4%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>3 (21.4%)</td>
<td>1 (5.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>Race:</strong></td>
<td>n/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White/Caucasian</td>
<td>10 (71.4%)</td>
<td>12 (66.7%)</td>
<td></td>
</tr>
<tr>
<td>Black/African American</td>
<td>2 (14.2%)</td>
<td>5 (27.8%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2 (14.2%)</td>
<td>1 (5.5%)</td>
<td></td>
</tr>
</tbody>
</table>
Human Microbiome: Methods

Sample: Mucosal biopsy, Stool swab, Fecal aspirate

DNA Extraction

Broad-Range PCR (bacterial 16S rRNA gene, V4 region, 250bp)

Illumina–based Sequencing (Miseq platform)

Q-PCR

Data Analysis:
• Taxonomic Identification
• Diversity analysis
• PCA
• Disease Correlations

Microbiome

(Explicit, R, Bray-Curtis for PCoA)
Mucosal Microbiome (Biopsies): Abundance at the Phylum Level

**Uninfected subjects**
- 51.3%
- 32.7%
- 7.1%

- Actinobacteria
- Bacteroidetes
- Cyanobacteria
- Firmicutes
- Fusobacteria

**HIV-infected subjects**
- 35.4%
- 33.0%
- 20.9%

- Proteobacteria
- Spirochaetes
- Tenericutes
- Verrucomicrobia
- Unclassified

*Phylum differences not seen in stool samples*
Abundance at the Genus Level: Increased Prevotella, Decreased Bacteroides in Colonic Mucosa

*Note: Differences in Prevotella and Bacteroides abundance at the genus level were also found in stool samples.
Principal Coordinates Analysis: Reducing the Dimensions of a Complex Dataset

PC1 and PC2 values for uninfected and HIV-infected subjects are shown. The p-value of 0.01 indicates a statistically significant difference between the groups.
PC1 associations: Bacterial families

More abundant
Lachnospiraceae
Bacteroidaceae
Prevotellaceae
Ruminococcaceae
Veillonellaceae
Helicobacteraceae
Campylobacteraceae
Porphyromonadaceae
Comamonadaceae
Moraxellaceae

Less abundant
Phylum:
Firmicutes
Proteobacteria
Bacteroidetes
Microbiome Associations with Immune Parameters
Dysbiosis in HIV infection was associated with:

Positive associations
- Plasma LPS levels
- Activated colon CD4 and CD8 T cells
- IFN-γ-producing colon CD8 T cells
- Activated colon Dendritic Cells (DC)
- Activated blood CD4 and CD8 T cells

Negative associations
- Number of colon Th22 cells (trend p=0.05)

* Based on PC analysis
**Prevotella** is associated with mucosal T cell activation in HIV+ subjects

**Activated colon CD4 T cells**

- Uninfected vs. HIV-infected
- Relative abundance of *Prevotella* (% of total bacteria)

**Activated colon CD8 T cells**

- Uninfected vs. HIV-infected
- Relative abundance of *Prevotella* (% of total bacteria)
*Prevotella* is associated with mucosal DC activation in HIV+ subjects

Activated colon mDCs

*Mucosal DC activation positively associated with mucosal and systemic T cell activation, mucosal viral load, mucosal cytokine production.*

Dillon et al., Mucosal Immunology, in press
Conclusions:

1) The HIV-related mucosal microbiome in untreated subjects was characterized by increases in *Prevotella* sp. and *Proteobacteria* and decreases in *Bacteroides* sp. and *Firmicutes* (*mucosa > stool*).

2) HIV-associated dysbiosis was linked to microbial translocation and to mucosal and systemic T cell activation.

3) Increased *Prevotella* abundance was most closely associated with immune activation.
Questions raised by results:

- Is HIV-associated pathology related to an increase in inflammatory or a decrease in regulatory bacteria?
- Is Prevotella a pathobiont?
  - Associated with periodontal disease, increased in lingual microbiome of HIV+ subjects
  - Can degrade mucin
  - Shown to promote blood DC activation and T cell IFN-γ production \textit{in vitro}
  - Associated with increased levels of atherogenic TMAO
- Some Bacteroides family members induce anti-inflammatory Tregs and IL-10.
- Firmicutes family members are known to be probiotics or to produce SCFA that contribute to epithelial barrier function and suppress inflammation
Short Chain Fatty Acids and Gut Homeostasis

- Short chain fatty acids (SCFAs) are metabolic products of dietary fiber fermentation by anaerobic bacteria of the large intestine.
- The human gut primarily contains acetate, propionate and butyrate (3:1:1 molar ratio).
- SCFAs act as cellular nutrients, pH modifiers, HDACi, GPCR agonists, and regulators of lipid and glucose metabolism.
- SCFA, especially butyrate, have immune modulating properties.
- Most butyrate is produced by Firmicutes (Clostridia class) in the human colon.

Lee et al., Nature Chemical Biology, 2014
Butyrate-producing Bacteria (BPB) are reduced during HIV infection

Notes:
• Based on Louis & Flint, FEMS Micr LTRs, 2009
• Based on 15 bacterial species isolated from human colon
Ratio of *Prevotella* to BPB species is increased during HIV infection

Mucosal *Prevotella*:BPB

![Graph showing the ratio of Prevotella to BPB species in Uninfected and HIV-infected subjects. The P-value is 0.002.](image)
Prevotella:BPB ratio is associated with Th17/Th22 Depletion and DC activation

*These data link dysbiosis to mucosal Th cell depletion. More in vitro work to come…
“Dual-Hit Hypothesis” for Dysbiosis–induced pathogenesis
Discussion Points

- What factors initiate and sustain dysbiosis during HIV infection? (i.e. inflammatory signals from epithelial and mucosal immune cells)
- What is the role of diet in pathogenesis in the setting of an altered microbiome?
- Which bacterial metabolites are altered during HIV infection? Which are most critical to inflammation?
- Are there other host factors that synergize with microbiome changes to worsen clinical outcomes? How do we design clinical studies to answer these questions?
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