HIV, Aging, and the Gut Microbiome: Inflammatory Consequences of Dysbiosis

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*No relevant COI to disclose for this presentation
The age of persons living with HIV is increasing: Convergence of age- and HIV-related Co-morbidity

Effros et al; CID 2008
HIV Infection and Aging Share Features of Inflammation and Immune Activation

- Increased pro-inflammatory cytokines and acute phase reactants (IL-6, TNF-a, CRP)
- Elevated levels of pro-coagulant factors
- Increase in cellular and soluble markers of monocyte activation (CD16+ monocytes, sCD14, sCD163)
- Increased markers of T cell activation and immune senescence
- Shared clinical outcomes include: dementia, CVD, strokes, metabolic alterations, frailty and sarcopenia, impaired immune responses and immune surveillance, cancers

Reviewed in Aberg, Top Antivir Med 2012
Are there common mechanisms driving inflammation in HIV infection and aging?
HIV Is a Mucosal Pathogen

HIV-1 infection disrupts intestinal immune homeostasis

- High levels of HIV replication in GALT
- Mucosal inflammation with aberrant cytokine production
- Immune activation (T cell, Dendritic cell, NK cell)
- Depletion of CD4 T cells
  - Th17, Th22 subsets
- Epithelial barrier disruption ("leaky gut")

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
Persistence of Gut Abnormalities Despite Viral Suppression

• Gut CD4 restoration and normal intestinal homeostasis are slowly and incompletely restored despite viral suppression on ART initiated during chronic infection (Kim JI 2013; Chege AIDS 2011; Guadalupe JV 2006).

• Early initiation of ART may preserve mucosal Th17 cells and reverse immune activation (Schuetz PLOS Path 2014).

• Microbial translocation persists despite effective ART and is associated with poor immune recovery (Lederman JID 2011).

• Markers of gut inflammation (iFABP) and microbial translocation (sCD14, LPS) are associated with non-AIDS adverse outcomes and death (Hunt JID 2014; Sandler JID 2011).
Questions:

- Is normal aging associated with intestinal inflammation and microbial translocation?
- Do increased systemic microbial products contribute to age-associated inflammation (aka “inflammaging”)?
- How do inflammaging profiles based on plasma biomarkers compare to HIV-associated inflammatory profiles?
# Cross-sectional HIV and Aging Study

<table>
<thead>
<tr>
<th></th>
<th>Uninfected participants</th>
<th>HIV-1 infected participants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of subjects</strong></td>
<td>88</td>
<td>83</td>
</tr>
<tr>
<td><strong>Male/Female</strong></td>
<td>55/33</td>
<td>69/14**</td>
</tr>
<tr>
<td><strong>Age (yrs) (median, range)</strong></td>
<td>38.5 (20-100)</td>
<td>56 (24-81)***</td>
</tr>
<tr>
<td>18-39yrs</td>
<td>27 (20-39)</td>
<td>32 (24-39) **</td>
</tr>
<tr>
<td>40-59yrs</td>
<td>50 (40-57)</td>
<td>49 (40-58)</td>
</tr>
<tr>
<td>60+yrs</td>
<td>69.5 (60-100)</td>
<td>64 (60-81) **</td>
</tr>
<tr>
<td><strong>HIV Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral Load (copies/mL)</td>
<td></td>
<td>&lt;48</td>
</tr>
<tr>
<td>CD4 T cell count (cells/mL)</td>
<td></td>
<td>595 (130-1400)</td>
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<tr>
<td>Infection duration (months) (n=76)</td>
<td></td>
<td>107 (14-303)</td>
</tr>
<tr>
<td>Time since first exposure to cART (months) (n=65)</td>
<td></td>
<td>77 (10-280)</td>
</tr>
</tbody>
</table>

Values shown as median (range). Non-parametric statistics; **p<0.01; ***p<0.0001;

Steele et al., PLOS ONE, May 2014
Study Outcome Measures

**Plasma Biomarker**
- Intestinal Fatty Acid Binding Protein (I-FABP)
- IL-6, hsCRP
- LPS (endotoxin)
- sCD14
- sCD27

**Biologic measure**
- Intestinal Epithelial Barrier Damage
- Systemic Inflammation
- Direct Microbial Translocation (MT)
- Monocyte activation, Indirect MT
- T cell activation
Physiologic Aging is associated with increased inflammation and immune activation

\[ r = 0.646 \quad p < 0.0001 \]

\[ r = 0.632 \quad p < 0.0001 \]

\[ r = 0.283 \quad p = 0.009 \]

\[ r = 0.553 \quad p < 0.0001 \]
Physiologic Aging is associated with increased Microbial Translocation and Gut Epithelial Barrier Damage

*I-FABP and LPS levels directly correlated with markers of monocyte and T cell activation but not inflammation*
Age-associated increases in iFABP and LPS were not seen in HIV-infected cohort.
Ischemic stress from hypoperfusion
Changes in Epithelial homeostatic signals (i.e. TLRs, NLRs)
Lamina Propria Inflammation
Altered Enteric Microbiome or “Dysbiosis”
Loss of mucin layer
Decrease in antimicrobial peptides
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

Characterization of Gut Bacterial Diversity in Untreated HIV-1 Infection: Clinical Study Design

Enrolled: 17 untreated HIV-infected and 14 seronegative control participants (Ages 18-55)

- Blood samples
  - CD4 count
  - Plasma HIV-1 RNA
  - T cell activation
  - Plasma biomarkers

- Study-specific interview
  - Clinical History
  - Dietary questionnaire

- Colon biopsies (flexible sigmoidoscopy)
  - Mucosal T cell and DC subset activation and cytokines
  - Tissue HIV-1 viral load
  - Microbiome analysis (substudy)

- Stool samples (rectal swabs)
  - Microbiome analysis

* Dillon SM, Mucosal Immunology, 2014.
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)
Altered Mucosal Microbiome: Abundance at the Phylum Level

Uninfected subjects

- Actinobacteria: 51.3%
- Bacteroidetes: 7.1%
- Cyanobacteria: 32.7%
- Firmicutes
- Fusobacteria

HIV-infected subjects

- Actinobacteria: 33.0%
- Bacteroidetes: 35.4%
- Cyanobacteria: 20.9%
- Firmicutes
- Fusobacteria

* Phylum differences not seen in stool samples
Within the Bacteroidetes Phylum: Increased Prevotella, Decreased Bacteroides in Colonic Mucosa

*Note: Differences in Prevotella and Bacteroides abundance at the genus level were also found in stool samples*
Butyrate-producing Bacteria (BPB) are reduced during HIV infection

MUCOSA

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

$P = 0.002$
Dysbiosis and host-microbe interactions: a balancing act

HIV-associated colonic dysbiosis at the Family taxa

Increase in bacteria with reported “pathogenic” properties (pathobionts) e.g. Prevotellaceae

Decrease in bacteria with anti-inflammatory properties e.g. Firmicutes: known to contain species of bacteria that produce short chain fatty acids (SCFAs)

Dillon et. al., Mucosal Immunology 2014
Diet Drives Gut Bacteria: “Enterotypes”

Enterotype is dependent primarily on long-term diet

Wu et al., Science 2011

Animal protein, saturated fat

Plant-based nutrition; high carbohydrate, low meat/dairy
Do dietary differences account for microbiome differences?

Closed symbols: Uninfected
Open symbols: HIV-infected
Disruption of *Bacteroides*: Red meat association in HIV Infection

Uninfected subjects

HIV-infected subjects

Mucosal immune factors trump dietary factors!
An altered gut microbiome is linked to MT and immune activation

- In our study, a Prevotella-rich, Firmicutes-poor dysbiosis was associated with:
  - Mucosal (colon) DC and T cell activation
  - Systemic T cell activation
  - Microbial translocation (LPS levels)

*Inflammation largely driven by Prevotella abundance

Dillon et al., Mucosal Immunology 2014
Prevotella:BPB ratio is associated with Th17/Th22 Depletion

*These data link dysbiosis to mucosal Th cell depletion, a characteristic feature of HIV pathogenesis.
“Dual-hit Hypothesis” for Dysbiosis-induced Pathogenesis

HIV-associated intestinal inflammation is a result of both increased exposure to bacteria with “pro-inflammatory” properties and a loss of immuno-regulation due to lower abundance of intestinal BPB (and lower tissue butyrate levels).
Gut Microbiome Changes with Age

- Increased inter-individual variability
- Decreased stability and biodiversity
- Increased abundance of gram negative facultative anaerobes (i.e. Enterobacteria) with pathogenic potential = pathobionts
- Decreased Firmicutes abundance and decreased Firmicutes:Bacteroidetes (Claesson 2011)
- Decreased SCFA-producing bacteria and decreased SCFAs in stool
- Decreased Bifidobacteria and Lactobacillus

*Results vary depending on age of cohorts, living situation, diet

Questions and Future Directions:

- How does the convergence of older age and chronic HIV infection impact the microbiome?
- What mucosal factors initiate and sustain dysbiosis during HIV infection and aging? (i.e. mucosal inflammation, hypoxia)
- Which bacterial metabolites are altered during HIV infection and aging? Which are most critical to inflammation?
- How does diet influence the gut microbiome and inflammation in aging HIV-infected individuals?
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