Dysbiotic microbes shaping immunity: Implications for HIV Transmission and Pathogenesis

Nichole Klatt
IMPAACT Meeting
January 25th, 2017
Vaginal Inflammation is a Key Factor in HIV Transmission

Innate Immune Activation Enhances HIV Acquisition in Women, Diminishing the Effectiveness of Tenofovir Microbicide Gel

Vivek Narambhai,1,2 Salim S. Abdool Karim,1,3 Marcus Altfeld,2,4 Natasha Samsunder,1 Raveshni Durgiah,2 Sengeziwe Sibeko,1 Quarraisha Abdool Karim,1,3 William H. Carr,2,4 and the CAPRISA004 TRAPS team

<table>
<thead>
<tr>
<th>Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td>TNF-α</td>
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<td>IL-8</td>
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<td>IL-5</td>
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<td>IL-6</td>
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<td>IL-1β</td>
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<td>IL-4</td>
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<td>IFN-γ</td>
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<td>IL-13</td>
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<td>IL-12</td>
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<td>IL-7</td>
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<td>IL-2</td>
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<td>IL-10</td>
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<td>GM-CSF</td>
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</table>
Bacterial Vaginosis (BV)

- Most common cause of vaginitis
- Loss of Lactobacillus spp. and increased diversity of vaginal microbiome
  - Gardnerella vaginalis, Atopobium vaginae, Prevotella spp., BV-associated bacteria (BVAB), etc.
- Vaginal inflammation, discharge, discomfort
- Associated with increased HIV risk
  - Male to female
  - Female to male
  - Mother to child

### Table 4. Wet Mount - Nugent’s Scoring

<table>
<thead>
<tr>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nugent score = 0</td>
<td>Nugent score = 10</td>
</tr>
</tbody>
</table>

- **Normal**
  - Lactobacilli common
  - Few to no other organisms

- **Abnormal**
  - Polymicrobial preparations
  - Clue cells
  - Coccobacilli, Gram variable
  - Lactobacilli few or absent
Vaginal microbiome diversity has been described by 16S rRNA sequencing.

Srinivasan et al. 2012

Anahtar et al., Immunity, 2015

Young women in SA have high vaginal microbial diversity.

Lack of functional information.
Antimicrobial functions of neutrophils

Phagocytosis, Degranulation, NETs

Anti-microbial functions

Tissue Damage
T cell suppression

The role of neutrophils in immune dysfunction during severe inflammation


Published: 23 March 2016
Model of mucosal inflammation in the FGT

(Burgener/McKinnon Mucosal Immunol 2016)
Neutrophil proteases are associated with pathogenic bacterial species in the FGT.
Do dysbiotic vaginal bacteria affect wound healing immune pathways?

Whole blood

Incubate with bacteria 14 hours

Associated with increased HIV transmission and BV:
*Gardnerella vaginalis*

Healthy bacteria:
*Lactobacillus crispatus*
*Lactobacillus iners*

Controls:
No stim (-)
Media alone (-)
LPS (+)
PGN (+)
Dysbiotic Vaginal Bacteria increase suppressive neutrophils

Non-stimulated

Stimulated with LPS

Stimulated with *Gardnerella vaginalis*

**MDSCs ("Suppressive" Neutrophils)**

- CD16^high^CD62L^low^ Neutrophils
  - No stimulation: 14%
  - + LPS: 74.2%
  - + *G. vaginalis*: 70.7%

- %CD16^high^CD62L^low^ Neutrophils:
  - No Stim: 50 ± 10
  - LPS: 90 ± 15
  - G. vaginalis: 80 ± 10
  - Lactobacillus: 60 ± 10

**"Normal" Neutrophils**

- PD-L1^+^ Neutrophils
  - No Stim: 20 ± 5
  - LPS: 40 ± 10
  - G. vaginalis: 30 ± 5
  - Lactobacillus: 10 ± 2

- %PD-L1^+^ Neutrophils:
  - No Stim: 0.05
  - LPS: 0.10
  - G. vaginalis: 0.08
  - Lactobacillus: 0.02

- p-values:
  - %CD16^high^CD62L^low^ Neutrophils: p = 0.0312
  - %PD-L1^+^ Neutrophils: p = 0.0002
Dysbiotic Vaginal Bacteria Promote Neutrophil Survival

No Stimulation

+LPS

+Liners

+G vaginalis

Caspase-3

Caspase3$^{\text{low}}$ (Neutrophil Survival)

\[ \% \text{Caspase3}^{\text{low}} \text{ Neutrophils} \]

\[ p = 0.0002 \]
Menstrual Cycle
Study Overview

- Pigtail macaques
  - Hormonal cycle similar to humans (~30 days)
  - Sex skin allows easy tracking of cycle

- Vaginal swabs from cycling female PTM in WaNPRC breeding colony
  - Cross sectional and Longitudinal

- Vaginal microbiome analysis
  - Broad range 16S rRNA gene PCR and pyrosequencing
  - Genus and species of vaginal bacteria

- Vaginal inflammation
  - High sensitivity NHP-specific luminex

- Vaginal immunity
  - Flow cytometry
Divergent vaginal bacteria throughout cycle and longitudinally

Rank Abundance

Macaque KO3541
- Prevotella copri
- Bacteroides/Prevotella
- Clostridiales Bacteroidetes
- Lactobacillus amylovorus
- Aerococcus
- Bacteroidales
- Streptococcus agalactiae
- Peptoniphilus harei
- Lactobacillales
- Streptococcus galolyticus

Macaque Z08151
- Fusobacterium naviforme
- Others

Follicular and Luteal

Relative % abundance (% 16S rRNA genes)

- Actinomycetales*
- Staphylococcaceae
- Bifidobacteriaceae
- Ruminococcaceae
- Clostridiales
- Eubacteriaceae
- Coriobacteriaceae
- Lactobacillales*
- Actinomycetaceae
- Bacilli**
- Corynebacteriaceae
- Veillonellaceae
- Campylobacteraceae
- Streptococcaceae
- Fusobacteriaceae
- Clostridiales*
- Lactobacillaceae
- Bacteroidaceae
- Porphyromonadaceae
- Leptotrichiaceae
- Prevotellaceae
- Peptoniphilaceae

0 10 20 30

Follicular
Luteal
Ovulation
Inflammation is altered throughout the cycle

**IL-12/23 (p40)**
- Follicular: p = 0.0088
- Ovulation: p = 0.0753

**TNF-a**
- Follicular: p = 0.0199
- Ovulation: p = 0.0477
Neutrophil function is altered throughout cycle
Innate inflammation is altered with microbiome

A10095

Vaginal IL-8

IL-8 pg/mL

BV Lacto Dominant

Z08165

Vaginal IL-1Ra

IL-1Ra pg/mL

BV Lacto Dominant

p = 0.0088

p = 0.0440
**Overlapping mechanisms underlying increased HIV transmission risk AND increased HIV pathogenesis**

<table>
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<td>1. Barrier Damage</td>
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Dysbiosis in HIV infection

Zevin, McKinnon, Burgener, Klatt; Curr Opinion HIV/AIDS 2016
Projected impact of highly active antiretroviral therapy on expected survival of a 20-year-old person living with HIV in a high-income country.
Inflammation (particularly associated with mucosal dysfunction) is a key predictor of morbidities and mortality.
Epithelial Barrier Breaches and Microbial Translocation

What are the mechanisms of reduced barrier integrity in HIV Infection?

Colon - Cytokeratin/E.coli/DAPI

SIV-

SIV+ (Non AIDS)

Estes et al., Plos Pathogens 2010
Immune Suppression by Neutrophils in HIV-1 Infection: Role of PD-L1/PD-1 Pathway

Nathan L. Bowers¹, E. Scott Helton¹, Richard P. H. Huijbrechts¹, Paul A. Goepfert²,³, Sonya L. Heath²,³, Zdenek Hel¹,²,⁴

Gut epithelial barrier and systemic inflammation during chronic HIV infection

Ma Somsouk¹, Jacob D. Estes¹, Claire Deleage², Richard M. Dunham³, Rebecca Albright³, John M. Inadomi⁴, Jeffrey N. Martin⁵, Steven G. Deeks⁶, Joseph M. McCune³ and Peter W. Hunt⁷

A. Myeloperoxidase (Neutrophils)
Question: How does altered microbiome in HIV infection affect neutrophil populations?

Whole blood

Incubate with HAMBs 20 hours

**Increased in HIV:**
- *Prevotella copri*
- *Prevotella stercorea*

**Decreased in HIV:**
- *Ruminococcus bromii*
- *Lactobacillus plantarum*

**Controls:**
- No stim (-)
- Media alone (-)
- LPS (+)

**Markers:**
- CD11b
- CD15
- SSC-A
- CD16
- CD62L
- PDL-1
- Arginase-1

**States:**
- "Suppressive"
- "Phagocytic"
Microbiota increased in HIV induce “suppressive” neutrophil phenotype, but not “normal” neutrophils.
Prevotella induce PD-L1 on neutrophils

**%PD-L1+ Neutrophils**

- **HIV-**
  - Media Only: 0%
  - LPS: 50%
  - Prevotella copri: 60%
  - Prevotella stercorea: 70%
  - Ruminococcus bromii: 80%
  - Lactobacillus plantarum: 90%

- **HIV+**
  - Media Only: 0%
  - LPS: 20%
  - Prevotella copri: 30%
  - Prevotella stercorea: 40%
  - Ruminococcus bromii: 50%
  - Lactobacillus plantarum: 60%

**PD-L1 MFI**

- **HIV-**
  - Media Only: 1000
  - LPS: 2000
  - Prevotella copri: 3000
  - Prevotella stercorea: 4000
  - Ruminococcus bromii: 5000
  - Lactobacillus plantarum: 6000

- **HIV+**
  - Media Only: 100
  - LPS: 200
  - Prevotella copri: 300
  - Prevotella stercorea: 400
  - Ruminococcus bromii: 500
  - Lactobacillus plantarum: 600

**Statistical Significance**

- **%PD-L1+ Neutrophils**
  - Media Only: p=0.0101
  - LPS: p=0.0014

- **PD-L1 MFI**
  - Media Only: p=0.0336
  - LPS: p=0.0043
  - Prevotella copri: p=0.0366
  - Prevotella stercorea: p=0.0366
  - Ruminococcus bromii: p=0.0366
  - Lactobacillus plantarum: p=0.0366
PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression


**PD-1+ T cells contribute significantly to SIV DNA pool**

PD-1 expression correlates with HIV load

PD-1 expression correlates inversely with CD4 count
Neutrophil PD-L1 expression associated with CD4+ T cell PD-1 expression

PD-L1+ Neutrophils vs PD1+ CD4 T Cells

$p=0.0065$
$r^2=0.1984$
Potential mechanism of neutrophil accumulation in GI tract is enhanced survival by dysbiotic bacteria

A. Myeloperoxidase (Neutrophils)

B. Caspase-3+ Neutrophils

Caspase-3

CD16

A. Media

HIV-

HIV+

B. LPS

Prevotella copri

Prevotella stercorea

Ruminococcus bromii

Lactobacillus plantarum

p=0.0014

p=0.0046

p=0.0024

p=0.0022
PD-L1 expression on neutrophils is associated with gut inflammation

MPO co-stains with neutrophils in GI tract
Can we exploit microbiome to benefit HIV prevention or pathogenesis?
Effects of Fecal Microbial Transplantation on Microbiome and Immunity in Simian Immunodeficiency Virus-Infected Macaques

Tiffany Hensley-McBain, Alexander S. Zevin, Jennifer Manuzak, Elise Smith, Jillian Gile, Charlene Miller, Brian Agricola, Michael Katze, R. Keith Reeves, Colleen S. Kraft, Stanley Langevin, Nichole R. Klett

Department of Pharmaceutics, University of Washington, Seattle, Washington, USA; Washington National Primate Research Center (WaNPRC), University of Washington, Seattle, Washington, USA; Department of Microbiology, University of Washington, Seattle, Washington, USA; Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA; Department of Pathology and Laboratory Medicine, Emory University Hospital, Atlanta, Georgia, USA.

A

B

Colon Genus Level 16S rRNA Sequences

Relative Abundance

Animal

Week -3 Pre-ABX

Week 2 Post-ABX

Week 6 Post-ABX

Week 1 Post-ABX

Week 5 Post-FMT

Week 3 Post-FMT

Flexispira

Helicobacter

Brachyspira

Prevotella

Succinivibrio

Morganella

Anaerovibrio

Anaeroplasma

Lactobacillus

Oscillospira

Bacteroides

Blautia

Clostridium

Providencia

Treponema

Campylobacter

Phascolarctobacterium

Sphingomonas

Escherichia

Mannheimia

Other
FIG 5: Correlation matrix of taxonomic groups that most strongly correlated with immunological or physiological parameters. Colors indicate Spearman correlation coefficient values ranging from −1 (dark blue) to 1 (dark red). The ellipse shape indicates the relative spread of data points and the slope of the correlation, with a wider ellipse indicating a greater relative spread and a narrower ellipse indicating less of a spread.
Therapeutic Interventions: Probiotics

• Microorganisms beneficial to the host

• Modulation of microflora
  • Inhibition of proinflammatory cytokines
  • Decreased intestinal permeability
  • Enhanced mucosal immunity

• Beneficial in many diseases: i.e. IBS, IBD, diarrheal diseases, respiratory diseases
Increased frequency of lymph node Tfh cells post-probiotic therapy
Increased frequency of IgA+ B cells in colon and LN post-probiotic therapy

Probiotic therapy down-modulates signaling through TLRs

Manuzak et al 2016
Persistent modulation of microbiota to enhance HIV vaccination
Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques

Nichole R. Klatt, Lauren A. Canary, Xiaoyong Sun, Carol L. Vinton, Nicholas T. Funderburg, David R. Morcock, Mariam Quiñones, Clayton B. Deming, Molly Perkins, Daria J. Hazuda, Michael D. Miller, Michael M. Lederman, Julie A. Segre, Jeffrey D. Lifson, Elias K. Haddad, Jacob D. Estes, and Jason M. Brenchley
ACTG Clinical Trial in HIV-infected individuals
Microbiome interventions for transmission?

- Interactions between microbiota and epithelium
- Interactions between microbiota and PrEP
- (Stay Tuned!)
Conclusions

• Microbiota can have direct effects on immune cells (esp. neutrophils), epithelial wound healing, and health

• Microbiota alter across cycle and may contribute to changes in inflammation

• Precise mechanisms by which microbiota induce disease or alter transmission need to be understood

• Implications for novel strategies to prevent and treat HIV infection
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  - Kenzie Birse
  - Laura Romas
  - Michelle Perner

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