Dynamic Vaginal Microbiota in Macaques Associated with Menstrual Cycle and Inflammation

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Vaginal Inflammation is a Key Factor in HIV Transmission

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

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Model of mucosal inflammation in the FGT

Slide courtesy of Adam Burgener, University of Manitoba
What impacts vaginal inflammation?

- Sexually Transmitted Infections (STIs)
- Bacterial Vaginosis (BV)


The Role of Bacterial Vaginosis and Trichomonas in HIV Transmission Across The Female Genital Tract

Paria Mirmontsef, Laurie Krass, Alan Landay, and Gregory T. Spear*

- Menstrual cycle/hormonal alterations

High Susceptibility to Repeated, Low-Dose, Vaginal SHIV Exposure Late in the Luteal Phase of the Menstrual Cycle of Pigtail Macaques

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BV is associated with loss of Lactobacillus and increased bacterial diversity

Figure 3. Representative vaginal microbiome data from women with (red) or without (green) BV (Srinivasan and Fredricks et al., Plos One 2012)
Question:
What is the nature of the vaginal microbiota of pigtail macaques relative to inflammation and 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 (N = 22)
  • Longitudinal (N = 3)

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

• Vaginal inflammation
  • High sensitivity NHP-specific luminex
Many vaginal bacteria are similar between human and pigtail macaques

- *Gardnerella vaginalis*
- *Atopobium vaginae*
- BV-Associated Bacterium 2 (BVAB2)
- *Prevotella timonensis*
- *Prevotella disiens*
- *Prevotella buccalis*
- *Mobiluncus mulieris*
- *Peptoniphilus lacrimalis*
- *Peptoniphilus harei*
- *Parvimonas micra*
- *Porphyromonas asaccharolytica*
- *Peptostreptococcus anaerobius*
- *Fusobacterium gonidiaformans*
- *Fusobacterium nucleatum*
- *Moryella indoligenes*
Diverse bacteria communities in vagina throughout menstrual cycle

Follicular

(Representative figures)
Diverse bacteria communities in vagina throughout menstrual cycle

Luteal

(Representative figures)
Lactobacillus dominant microbiome observed at ovulation

Ovulation

(Representative figures)
Divergent bacterial communities throughout cycle within same animal sampled longitudinally.
Lactobacillus dominant vaginal microbiota was associated with decreased innate inflammation in the vagina.
Inflammatory markers across cycle

- Inflammatory cytokines similar in follicular vs luteal, but higher than ovulation
- Follicular increases anti-inflammatory cytokine
- What accounts for decreased inflammation during ovulation?
Neutrophil function is altered throughout cycle

Vaginal Neutrophils

Blood Neutrophil Functionality

% CD3/CD11b+CD14+/HLA-DR

p = 0.0309

p = 0.0068

p = 0.0431

% phagocytosis

Stage of Cycle

Follicular
Ovulation
Luteal
Conclusions I - Macaque Microbiota

• Pigtail macaques had diverse bacterial communities at all phases of the menstrual cycle, with a range of dominant bacteria in different animals
  – *Prevotella*, *Porphyromonas*, *Dialister*, *Streptococcus* and *Lactobacillus* species.

• 3 animals had *Lactobacillus*-dominant vaginal microbiota
  – all at peak sex skin swelling (indicating ovulation)

• Several bacteria were identified that are commonly found in bacterial vaginosis (BV) in humans
  – *Atopobium vaginae*, *Prevotella buccalis*, BV-associated bacterium-2 (BVAB2), *Sneathia*, *Peptoniphilus lacrimalis*, *Prevotella timonensis* and *Gardnerella vaginalis*

• Longitudinal sampling demonstrated that vaginal bacterial communities were dynamic and possibly influenced by alterations in environment throughout the menstrual cycle
Conclusions II - Inflammation

• Inflammatory cytokines in PTM vagina were increased in follicular and luteal compared to ovulatory
  – Decreased regulatory cytokines at luteal

• Lactobacillus-dominant microbiota was associated with decreased innate inflammation
  – Associated with frequency of *Lactobacillus*

• Neutrophil frequency increased but function decreased in luteal phase
Interpretations and Future Directions

• Importance of healthy microenvironment in the vagina to protect from HIV infection
• Full longitudinal study to understand dynamic bacteria throughout cycle
• Can we alter the vaginal microenvironment to favor decreased HIV susceptibility?
  – Antibiotics (Metronidazole)
  – Seeding with human bacteria, probiotics
  – Altering neutrophil responses
• Better understanding how neutrophils alter epithelial barrier and drive inflammation
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