Male Genital Tract Microbiota: Insights into HIV Acquisition

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Male Circumcision

Circumcision reduces HIV-1 acquisition by 60%

Still don’t really understand how
Hypothesis

- BV in women → Genital anaerobes = HIV susceptibility
- Mediated by inflammation?
Methods

• Swab samples of coronal sulcus (microbiome, soluble immune markers)

• Foreskin tissue collection (model for mucosal immune responses)

• Microbiome
  – 16S rRNA gene-based quantitative PCR and pyrosequencing

• Soluble chemokines
  – Multiplexed ELISA (MesoScale Discovery)
Circumcision reduces prevalence and load of penile anaerobic bacteria

- MC decreased total bacterial load
- MC decreased microbiota diversity
  - Slight increase in aerobic taxa
  - Prevalence and absolute abundance of 12 bacterial taxa significantly decreased
Circumcision decreases penile IL-8

<table>
<thead>
<tr>
<th></th>
<th>% Detectable</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>59.4</td>
</tr>
<tr>
<td>MIG</td>
<td>25.0</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>6.7</td>
</tr>
<tr>
<td>MCP-1</td>
<td>6.7</td>
</tr>
<tr>
<td>MIP3α</td>
<td>5.0</td>
</tr>
<tr>
<td>IL-1α</td>
<td>3.9</td>
</tr>
<tr>
<td>RANTES</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Prodger 2016 Plos Path
Testing our hypothesis:

• Nested case-control study of seroconverters:
  – 60 seroconverters, 120 control (all uncircumcised)
Higher absolute abundance of anaerobic bacteria associated with seroconversion

• Remains significant after controlling for STIs and other demographics

Liu 2017 mBio
### Cytokines associated with seroconversion

<table>
<thead>
<tr>
<th>Detectable Cytokine</th>
<th>Controls n=120</th>
<th>Seroconverters N=60</th>
<th>OR (95% CI)</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>63 (52.5%)</td>
<td>44 (73.3%)</td>
<td>2.52 (1.28, 4.99)</td>
<td>2.26 (1.04, 6.40)</td>
</tr>
<tr>
<td>MIG</td>
<td>23 (19.7%)</td>
<td>22 (36.7%)</td>
<td>2.49 (1.23, 5.03)</td>
<td>2.72 (1.15, 8.06)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>5 (4.2%)</td>
<td>7 (11.7%)</td>
<td>3.02 (0.92, 9.91)</td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>6 (5.0%)</td>
<td>6 (10.0%)</td>
<td>2.10 (0.65, 6.79)</td>
<td></td>
</tr>
<tr>
<td>MIP3α</td>
<td>4 (3.3%)</td>
<td>5 (8.3%)</td>
<td>2.61 (0.68, 10.06)</td>
<td></td>
</tr>
<tr>
<td>IL-1a</td>
<td>4 (3.3%)</td>
<td>3 (5.0%)</td>
<td>1.53 (0.33, 7.16)</td>
<td></td>
</tr>
<tr>
<td>RANTES</td>
<td>3 (2.5%)</td>
<td>2 (3.3%)</td>
<td>1.35 (0.22, 8.30)</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for STI diagnostics (syphilis and HSV-2), and all variables associated with either seroconversion, IL-8 or MIG (occupation, marital status, multiple sex partners, condom use, alcohol use, STI symptoms)*

Prodger 2016 Plos Path
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</thead>
<tbody>
<tr>
<td>0</td>
<td>55 (45.8%)</td>
<td>14 (23.3%)</td>
<td>REF</td>
<td>REF</td>
</tr>
<tr>
<td>1</td>
<td>42 (35.0%)</td>
<td>25 (41.7%)</td>
<td>2.34 (1.09, 5.03)</td>
<td>2.56 (0.93, 7.70)</td>
</tr>
<tr>
<td>2+</td>
<td>23 (19.2%)</td>
<td>21 (35.0%)</td>
<td>3.78 (1.61, 8.90)</td>
<td><strong>3.30 (1.21, 12.50)</strong></td>
</tr>
</tbody>
</table>

*Adjusted for STI diagnostics (syphilis and HSV-2), and all variables associated with either seroconversion, IL-8 or MIG (occupation, marital status, multiple sex partners, condom use, alcohol use, STI symptoms).*

Prodger 2016 Plos Path
Anaerobes and IL-8

Liu 2017 mBio
IL-8 and MIG Associated with Increased Neutrophils in Foreskin

CD15+ neutrophils in the foreskin highlighted in green by immunofluorescence

Prodger 2016 Plos Path
Anaerobe species correlate with HIV target cells

**Peptostreptococcus anaerobius**
- rho = 0.396, p < 0.001

**Dialister propionicifaciens**
- rho = 0.280, p = 0.009

**Dialister succinatophilus <0.97**
- rho = 0.227, p = 0.035

**Dialister micraerophilus**
- rho = 0.276, p = 0.010

**Prevotella bivia**
- rho = 0.399, p < 0.001

**Prevotella disiens**
- rho = 0.345, p = 0.001

**Prevotella disiens <0.97**
- rho = 0.220, p = 0.042

**Staphylococcus spp.**
- rho = -0.018, p = 0.9

CCR5+ CD4 T cells (log10 cells/mm²)

Log₁₀ Absolute abundance
Summary

- Male circumcision reduces penile anaerobes & IL-8
- Increased penile cytokines are associated with subsequent HIV seroconversion
- Penile anaerobes associated with increased penile IL-8 levels
- IL-8 associated with increased density of foreskin CCR5+ CD4 T cells and neutrophils in the foreskin
- Circumcision may protect against HIV by reducing local immune activation through the elimination of key anaerobes
Conclusions and Future Directions

• The foreskin provides a unique opportunity to understand mucosal immunology and HIV acquisition.

• Further understanding of penile inflammation is needed to develop novel mechanisms to reduce HIV acquisition and transmission.
  – Using discovery-based analysis, evaluating what penile bacterial species associated with seroconversion
  – Assessing whether reproductive maturation and sexual debut in adolescent boys leads to changes in the penile microbiome that affects the genital immunological milieu and risk of acquiring HIV
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