Why STI-associated genital tract inflammation still matters in HIV transmission

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A healthy female genital mucosa presents a relatively effective barrier to HIV entry during sex

Miller et al. 2005

Protection by mucus barrier

Efficiency of "transgressing" mucosal barrier low with only few foci of virus detected

Window of opportunity:
6 days to intervene!

How does genital tract inflammation place women at higher risk for HIV infection?

Li et al. 2009
Haase et al. 2010
Haase et al. 2012

The battlefield is fuelled by inflammation allowing spread of local genital tract infections to draining lymph nodes and then, systemically

Window of opportunity:
Manage inflammation (STIs) to block HIV infection!

Part 1

Women with genital tract inflammation (cytokines) prior to HIV infection were at increased risk for acquiring HIV

Women who later became HIV-infected had pre-infection genital inflammation

Dr Lindi Masson (UCT), Dr Lenine Liebenberg (CAPRISA)
Genital tract chemokines (MIP-1α, MIP-1β, IP-10, IL-8) were associated with HIV acquisition

Potential causes of genital inflammation in CAPRISA004

Genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study

Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study

Potential causes of genital inflammation in CAPRISA004

Microbiome analysis ongoing

Only 20% of HIV infections could be attributed to (or were a result of) an STI. T. vaginalis was the most strongly predictive of genital inflammation.

Dr Lyle McKinnon, CAPRISA – S14.3 11:30-11:45am Rm M3 Mezzanine

Potential causes of genital inflammation in CAPRISA004

Potential causes of genital inflammation in CAPRISA004

Microbiome analysis ongoing

Dr Lyle McKinnon, (and Adam Burgener)
Overlap between **genital** versus **plasma** cytokine signatures to predict HIV infection in CAPRISA004

No correlation between genital tract and plasma cytokines

<table>
<thead>
<tr>
<th>Plasma cytokine concentrations (pg/ml)</th>
<th>Spearman Rho</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.0084</td>
<td>0.93</td>
</tr>
<tr>
<td>IL-1α</td>
<td>0.1573</td>
<td>0.10</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.0140</td>
<td>0.87</td>
</tr>
<tr>
<td>IL-7</td>
<td>0.0717</td>
<td>0.46</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.0954</td>
<td>0.33</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.1272</td>
<td>0.19</td>
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<tr>
<td>GM-CSF</td>
<td>0.1020</td>
<td>0.29</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>0.0372</td>
<td>0.70</td>
</tr>
<tr>
<td>IP-10</td>
<td>0.0969</td>
<td>0.32</td>
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<tr>
<td>MCP-1</td>
<td>0.0366</td>
<td>0.71</td>
</tr>
<tr>
<td>MCP-1a</td>
<td>0.0610</td>
<td>0.53</td>
</tr>
<tr>
<td>MCP-1α</td>
<td>0.0290</td>
<td>0.79</td>
</tr>
</tbody>
</table>

**South African Press coverage about genital inflammation and HIV risk in women.**

Lindi Masson handling some very awkward questions about genital inflammation from the South African public in interviews on several radio news shows in June.

**Discussion (Part 1)**

- **Women who had genital inflammation were at increased risk of HIV infection**
- Elevated concentrations of 4 chemokines (MIP-1α, MIP-1β, IP-10 and IL-8) were associated with increased risk of HIV infection
- These chemokines are likely to recruit potential HIV target cells
- MIP-1β, in particular, bind to the HIV co-receptor CCR5 and specifically recruit CCR5+ HIV target cells that potentially enhance HIV infection.
- In macaques, production of these and other inflammatory cytokines has been shown to be essential for recruitment of CD4+ T cell targets needed for SIV replication

**Part 2**

Women who acquired HIV during CAPRISA004 had high frequencies of CD68+ and CD4+ target cells within the stratified squamous epithelium (from vaginal biopsies)

In collaboration with Prof Thomas Hope and Dr Ann Carias Northwestern University, Chicago

Dr Lenine Liebenberg, CAPRISA; S17.2 2.05-2.25pm M1-2, Mezzanine
CD4 target cell density in CAPRISA004 vaginal biopsies

Median density 30.5 (20-64) cells/mm²
Median depth 113 (62-189) um

CD88 cell density in CAPRISA004 vaginal biopsies

Median density 22.6 (16-30) cells/mm²
Median depth 156 (132-164) um

Relationship between target cell density in biopsies and genital tract (CVL) cytokines

CD88+ macrophage density in vaginal biopsies were positively associated with chemokines and growth factor concentrations in matching CVLs

Not so for CD4+ cells

Relationship between target cell depth in biopsies and genital tract (CVL) cytokines

The depth of CD4+ target cells in the vaginal epithelium was positively associated with chemokines and inflammatory cytokines in CVL

BUT depth of CD88+ target cells were associated negatively with inflammatory cytokines, chemokines and growth factors

Part 3

Exploring younger age and adolescence as important risk factors for HIV acquisition

EDCTP Mucosal Primer

The WISH study
(Women’s Initiative in Sexual Health)

Adolescent genital immune activation and inflammation

Study in 300 adolescent females (16-22 year olds) from Masipumelele, Cape Town (DTHF Youth Centre) and Soweto, Johannesburg (PHRU)
EDCTP Mucosal Primer
The WISH study
(Women’s Initiative in Sexual Health)

- Cytokines (luminex, 48, Biorad)
- T cell activation (cytobrush CD38, HLA-DR, CCR5, Ki67 on CD4+ and CD8+ by FACS, ex vivo)
- Microbiome (16S)
- Culture

Prevalence of STIs (particularly CT) and BV higher in adolescent women in Cape Town compared to Johannesburg, South Africa

<table>
<thead>
<tr>
<th>STI</th>
<th>Cape Town (%)</th>
<th>Johannesburg (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydia trachomatis</td>
<td>41.6% (62/149)</td>
<td>15% (15/100)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>7.4% (11/149)</td>
<td>4% (4/100)</td>
<td>0.2507</td>
</tr>
<tr>
<td>Neisseria gonorrhoea</td>
<td>11.4% (17/149)</td>
<td>4% (4/100)</td>
<td>0.0140</td>
</tr>
<tr>
<td>HSV-2</td>
<td>4.7% (7/149)</td>
<td>0% (0/100)</td>
<td>0.0289</td>
</tr>
<tr>
<td>Mycoplasma genitalium</td>
<td>4.0% (6/149)</td>
<td>2% (2/100)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Bacterial vaginosis (Nugent &gt;7)</td>
<td>48.0% (71/148)</td>
<td>31% (31/100)</td>
<td>0.0763</td>
</tr>
<tr>
<td>Total</td>
<td>150/150</td>
<td>100/150</td>
<td></td>
</tr>
</tbody>
</table>

Comparing the genital cytokines between Cape Town and Johannesburg

Unsupervised hierarchical clustering was used to visualize the variation in genital tract cytokine concentrations in individual women and to cluster women across 2 sites in South Africa according to the similarities of their cytokine expression profiles (using Quocore Omics Explorer).
Comparing the vaginal microbiome between Cape Town and Johannesburg in STI/BV negative adolescents

Longitudinal cytobrush CD4 T cell activation

T cell activation did not differ significantly between time points (spanning 4 months) T cell activation did not correlate significantly between visits

Relationship between genital tract cytokines and cervical T cell activation

Vaginal microbiome according to genital inflammatory cytokine score

Having an STI or BV (Nugent >7) increases the frequency of activated CD4 T cells in the female genital tract (cytobrush)

Relationship between cervical cytokines and cervical T cell activation

Genital inflammatory cytokine score

Inflammatory Chemokines Growth Factors/ Haematopoetic Adaptive Regulatory

Pre-inflammatory Cytokines Growth Factors/ Adaptive Anti-inflammatory
Discussion (Part 2)

• A collaborating network of South African clinical and laboratory investigators has been established, with specific expertise in mucosal assessment for conducting HIV prevention research

• Young women, particularly in Cape Town, had unacceptably high rates of asymptomatic STIs and BV, calling for an urgent re-evaluation of how appropriate our current STI surveillance and testing guidelines are for sub-Saharan Africa

• Both having an STI and/or BV was associated with significant increases in both inflammatory cytokines and chemokines in genital secretions and frequencies of activated CD4+ HIV target cells at the cervix

• This study provides an important link between genital cytokine markers of inflammation and cellular activation