Genital immunology and HIV transmission

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Key messages

- Low but very variable risk of HIV transmission after sexual exposure
- Mucosal immunology is important:
  - Number &/or nature of target cells in genital/rectal lining (if HIV-)
  - Antimicrobial defenses
- BUT it can be very difficult to define “protective” mucosal immune factors

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HIV transmission dichotomy

Adults and children estimated to be living with HIV, 2008

Table 2 The risk of HIV transmission following an exposure from a known HIV-positive individual

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>Estimated risk of HIV transmission per exposure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood transfusion (one unit)</td>
<td>90–100⁵</td>
</tr>
<tr>
<td>Receptive anal intercourse</td>
<td>0.1–3.0⁵,⁷</td>
</tr>
<tr>
<td>Receptive vaginal intercourse</td>
<td>0.1–0.2⁷,¹²</td>
</tr>
<tr>
<td>Insertive vaginal intercourse</td>
<td>0.03–0.09¹⁰</td>
</tr>
<tr>
<td>Insertive anal intercourse</td>
<td>0.06¹³</td>
</tr>
<tr>
<td>Receptive oral sex (fellatio)</td>
<td>0–0.04¹³</td>
</tr>
<tr>
<td>Needle-stick injury</td>
<td>0.3 (95 CI 0.2–0.5)¹⁴–¹⁶</td>
</tr>
<tr>
<td>Sharing injecting equipment</td>
<td>0.67¹⁷</td>
</tr>
<tr>
<td>Mucous membrane exposure</td>
<td>0.09 (95 CI 0.006–0.5)¹⁸</td>
</tr>
</tbody>
</table>


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HIV transmission requirements

• Infectious virus in genital/rectal secretions of the infected partner (semen)
• Access to susceptible cells in the exposed mucosa of the uninfected partner
• Avoidance of innate antimicrobial proteins: 
  – α/β defensins, SLPI, RANTES
  – Trappin-2 (elafin), cathelicidins
Transmission requires mucosal targets


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Target density and early infection

Day 4 pi  Day 7 pi

Day 7-10: virus detectable in peripheral blood.


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Target availability differs at mucosal sites of exposure

Expression of HIV-1 co-receptors on CD4 T cells in tonsillar and rectosigmoid tissues.


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Mucosal immune studies

- Cells collected by cytobrush
- Populations quantified by flow cytometry
- Genital secretions (CVL or Instead cup)
  - STI diagnostics
  - Cytokines, other innate immune factors

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- CD4+ T cells in the mucosa are likely to be central to HIV acquisition

- High levels of activated cells, also increased expression of HIV coreceptors

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Genital co-infections and genital immunology
Genital co-infections and HIV acquisition

- Genital infections associated with increased HIV incidence in numerous studies
  - HSV-2: ~3-fold increase (Glynn J. AIDS, 2009)
  - Bacterial vaginosis (BV): ~60% increase (Atashili J. AIDS, 2008)
  - Trichomoniasis, gonorrhea, etc: similar
Common mucosal immune effects:
increased CD4, dendritic cells

Shin L, unpublished.

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So why doesn’t STI therapy reduce HIV transmission?

• Population based STI prevention / therapy has generally had minimal impact
• Individual level STI prevention / therapy had no impact
• HSV-2 suppression has had no impact
  – ? Co-infection role in different stages of the epidemic
  – ? Delayed mucosal resolution after Rx
Delayed effects of HSV-2 therapy in reducing target cells


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Impact of treatment

- Took >2 weeks to see significant reduction

* Wilcoxon signed ranks sum
**Wilcoxon paired ranks test

Shin L, unpublished.

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Defining protective genital immune factors
## Genital defenses against HIV: *in vitro*

<table>
<thead>
<tr>
<th>Mucosal innate immune factor(s)</th>
<th>HIV effects <em>in vitro</em></th>
<th>Individuals/conditions with reduced HIV susceptibility&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLPI</td>
<td>↓ Replication&lt;sup&gt;24&lt;/sup&gt;</td>
<td>↑ (in infant saliva) reduced MTCT&lt;sup&gt;48&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trappin-2</td>
<td>↓ Replication&lt;sup&gt;51&lt;/sup&gt;</td>
<td>↑ (in maternal vagina) reduced MTCT&lt;sup&gt;47&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>↓ HIV replication; ↓ epithelial adsorption&lt;sup&gt;30&lt;/sup&gt;</td>
<td>↔ in HEPS&lt;sup&gt;49,50&lt;/sup&gt;</td>
</tr>
<tr>
<td>Defensins (α/β)</td>
<td>↓ Replication</td>
<td>↑ in HEPS FGT&lt;sup&gt;**51&lt;/sup&gt;</td>
</tr>
<tr>
<td>IFNa (type I interferons)</td>
<td>↓ Replication&lt;sup&gt;41,42&lt;/sup&gt;</td>
<td>↑ in HEPS cervix&lt;sup&gt;49,54&lt;/sup&gt;</td>
</tr>
<tr>
<td>β chemokines (RANTES, MIP1α, MIP1β)</td>
<td>↓ Replication</td>
<td>↑ in HEPS cervix&lt;sup&gt;49&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epithelial cell integrity</td>
<td>↓ HIV infectivity</td>
<td>↑/↔ in HEPS cervix&lt;sup&gt;49,50&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>b</sup> No data
“Real world” immune protection: harder to define

• Most exposure does NOT lead to infection:
  – Tiny minority of needle stick injuries, sexual contacts
  – Mean transmission within a discordant couple is ~12% per year (Quinn 2000)

• Need very high infection pressure to truly deem a person as highly exposed seronegative (HESN)
Female sex workers and HIV immune protection

• Female sex worker (FSW) cohorts
  – HIV incidence often high, so can identify HESN individuals
  – Examples: Pumwani, Kibera FSW communities

• Problems with such studies:
  – Incidence varies from cohort to cohort, so must be measured
  – Over time in same cohort
  – HIV status of clients is generally unknown

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Other confounding factors

• HESN sex workers have multiple partners
  – Eg: mean of 4-6 clients/day in Pumwani
• Substantial proportion must be unprotected to result in “threshold” infection pressure
• Frequent unprotected sex:
  – Semen, sex both induce immune alterations
  – Genital co-infections alter immunology
### HSV-2 is increased in HESN FSW

<table>
<thead>
<tr>
<th>Demographic or risk factor</th>
<th>Relatively resistant (N=80)</th>
<th>Not resistant (N=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean)</td>
<td>40.5</td>
<td>33.9***</td>
</tr>
<tr>
<td>Duration of cohort enrolment, years (mean)</td>
<td>9.0</td>
<td>1.7**</td>
</tr>
<tr>
<td>Duration of any sex work, years (mean)</td>
<td>13.8</td>
<td>5.9**</td>
</tr>
<tr>
<td>HSV-2 seropositive (n, %)</td>
<td>75/80 (94%)</td>
<td>46/59 (78%)**</td>
</tr>
<tr>
<td>Clients /week (mean)</td>
<td>21.7</td>
<td>21.4</td>
</tr>
<tr>
<td>Condoms /week (mean)</td>
<td>22.1</td>
<td>30.8</td>
</tr>
<tr>
<td>Bacterial vaginosis (n, %)</td>
<td>13/43 (30%)</td>
<td>18/74 (24%)</td>
</tr>
<tr>
<td>Syphilis</td>
<td>4/77</td>
<td>0/49*</td>
</tr>
</tbody>
</table>

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Studies must rule out confounders

• Full infection diagnostics
• Have an open mind: associations seen could either be:
  – The holy grail of protective immunity
  – A paraphenomenon of risk
• Ideally any associations should be confirmed prospectively…
Prospective immune correlates of HIV protection

• Studied in an RCT of bacterial STI prevention
• HIV-uninfected Kenyan sex workers (N=466)
• Cervicovaginal lavage samples banked at enrolment
• Women acquiring HIV (N=35) were matched with 4 controls who did not by independent biostatistician
• Blindly assayed genital levels of several antimicrobial peptides, HIV neutralizing effect of genital secretions
• Stratified case-control analysis

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Table 3. Neutralizing activity against clade A and clade C primary HIV isolates.

<table>
<thead>
<tr>
<th></th>
<th>Neutralizing$^a$</th>
<th>Not neutralizing$^a$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$(n = 13)$</td>
<td>$(n = 100)$</td>
<td></td>
</tr>
<tr>
<td>HNP-1-3</td>
<td>1096 (67–3888)</td>
<td>175 (0.05–4658)</td>
<td>0.007$^b$</td>
</tr>
<tr>
<td>SLPI</td>
<td>200 (51–602)</td>
<td>207 (15–587)</td>
<td>0.443</td>
</tr>
<tr>
<td>LL-37</td>
<td>27 (5–188)</td>
<td>10 (2–191)</td>
<td>0.002$^b$</td>
</tr>
<tr>
<td>HBD-2-3</td>
<td>7 (1–220)</td>
<td>5 (0.2–179)</td>
<td>0.247</td>
</tr>
<tr>
<td>RANTES</td>
<td>30 (5–1812)</td>
<td>5 (3–2027)</td>
<td>0.191</td>
</tr>
</tbody>
</table>

Levels of innate immune molecules as measured in the IgA1-depleted 5 ml cervicovaginal secretion were correlated to neutralizing activity against both a clade A and a clade C primary HIV isolate. HBD, human β defensin; HNP, human neutrophil peptide; RANTES, regulated upon activation normal T-cell expressed and secreted; SLPI, secretory leukocyte protease inhibitor.

$^a$Median (range).

$^b$Statistical significance.

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• \(\alpha\)-defensin & LL37 levels were higher in those who acquired HIV

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Sex Trans Infect; In Press.

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Genital immune factors and genital infections

Fig. 2. The presence of multiple ongoing infections was strongly associated with increased levels of human neutrophil peptide-1-3, human β defensin-2-3 and LL-37.

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SUMMARY

• HIV infectivity is surprisingly low, but very heterogeneous
• Requires susceptible cells in the mucosa for initial infection and local replication
• Genital co-infections have shared immune effects
  – Increase both genital HIV levels and target cells
  – BUT STI prevention has not impacted HIV acquisition
• Studies of “protective” immune factors must be careful to avoid confounding
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