Susceptibility of Epithelial Cells from the Female Upper Reproductive Tract to Infection by Transmitted/Founder HIV-1

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50% of infected adults, & 80% of new HIV cases in 2007, are women, and the HIV epidemic affects women mostly via sexual transmission.

In the majority of cases, infection is established by one viral genome.

Mechanisms of transmission in the female reproductive tract (FRT) remain poorly understood.

Upper FRT sites, including uterine endometrium, are only recently being recognized as potentially vulnerable sites for HIV-1 infection. They are not a sterile environment, and semen contents (sperm; pathogens; experimental dyes) reach the upper FRT quickly.

Immune functions of the FRT are under tight endocrine control and change profoundly throughout the menstrual cycle, possible creating a “Window of Vulnerability” (Wira and Fahey, 2008).
Uterine Epithelial Cell HIV Coreceptor Expression During Stages of the Menstrual Cycle

- UEC cells can express CD4, CXCR4, CCR5, and GalCer.
- thus, they may represent first target cells for infection.

HYPOTHESIS:
The upper FRT is a vulnerable site for HIV-1 transmission, and immune and epithelial cells comprising these tissues are susceptible to HIV-1 infection.

• Prior work on UEC has been somewhat inconclusive and was conducted with non-polarized cells and lab-adapted/reference strain, or chronic HIV-1.

APPROACH:
• Revisit the question using primary and immortalized uterine epithelial cells in their polarized state and employing bona fide mucosally transmitted HIV-1 (either IMC and reporter Env-IMC expressing transmitted and reference env).
Experimental approach:
Env-IMC-LucR viruses expressing T/F or reference strain envs

Key features of reporter Env-IMC-Luc viruses:
✓ expression of env in cis, and expression of all viral proteins,
✓ replication competent beyond a single cycle of infection,
✓ includes, and stably maintains, the luciferase reporter within the provirus,
✓ a molecular strategy to conveniently “shuttle in” heterologous env sequences,
✓ using primary cells, sensitive, quantitative detection of reporter gene activity with large dynamic range.
Examples of sensitive detection of infection with Env-IMC-LucR viruses in primary cells

- Low background: ~150 RLU;
- Linear dynamic range from 2x background to >10^6 RLU
- A - In Monocyte-derived macrophages, env-mediated differences in infection / replication kinetics are evident by LucR activity at 2 days p.i.
- B - Detection of single round infection of PBMC as early as 16 hours p.i.; estimated limit of detection in PBMC ~50 infected cells.

![Graph showing LucR activity](image1)

![Graph showing LucR activity over time](image2)
Are primary uterine epithelial cells (UEC) susceptible to HIV-1 infection mediated by transmitted / founder (T/F) env genes?
Epithelial Cell Preparation for Analysis of Susceptibility of Polarized Uterine Cells to HIV-1

1. Enzymatic digestion of FRT tissues to obtain epithelial & stromal cells
2. 2-step filtration to obtain UEC sheets

- Stromal Cell Suspension
- UEC are CD4+, CXCR4+, Gal Cer+, CCR5+
  - Expression levels vary throughout menstrual cycle
Polarized primary UEC are susceptible to apical HIV-1 infection mediated by T/F env genes (1)
Polarized primary UEC are susceptible to apical HIV-1 infection mediated by T/F env genes (2)

- The levels and pattern of reporter gene expression observed in cells from a total of 6 patients suggest that T/F viruses can productively infect primary UEC.
- As observed for other primary cell models (PBMC, macrophages, mucosal tissue explants), not all donors support HIV-1 infection equally well.
Can the susceptibility phenotype observed in polarized primary UEC be replicated with a uterine epithelial model cell line, ECC-1?

Polarized ECC-1 cells are CD4+, CXCR4+, Gal Cer+; CCR5 low/- ** (Asin et al., JID187 (2003))
Polarized ECC-1 are susceptible to apical HIV-1 infection mediated by T/F env genes (1)

ECC-1 on filters (n=3) Exp. 1

ECC-1 on filters (n=3) Exp. 2

3 x Blank-corrected Uninfected Average
Polarized ECC-1 are susceptible to apical HIV-1 infection mediated by T/F env genes (2)

ECC-1 on filters (n=3) Exp. 3

Apical infection

Basolateral infection

3 x Blank-corrected Uninfected Average
Additional T/F env genes, incl. those from female patients, mediate infection/ LucR expression in ECC-1, while BaL and SF162 env do not. (viral input normalized to equal i.u. & LucR RLU in TZMbl +/- DEAE dextran).
Infection of ECC-1 cells with GFP-reporter viruses expressing the transmitted and reference envs (Env-IMC-GFP)

<table>
<thead>
<tr>
<th>env gene</th>
<th>Reference strain or T/F</th>
<th>GFP+ cells</th>
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<tbody>
<tr>
<td>NL4-3</td>
<td></td>
<td>Generally zero, with rare (1 – 2) positive cells</td>
</tr>
<tr>
<td>BaL</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>SF162</td>
<td>T/F</td>
<td>10 – 20 positive cells</td>
</tr>
<tr>
<td>YU-2</td>
<td>T/F</td>
<td></td>
</tr>
<tr>
<td>JRCSF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH058</td>
<td>T/F</td>
<td></td>
</tr>
<tr>
<td>CH077</td>
<td>T/F</td>
<td></td>
</tr>
<tr>
<td>VSV-G pseudotyped</td>
<td>control</td>
<td>&gt;&gt;100</td>
</tr>
</tbody>
</table>

- use of an alternative reporter virus read-out, GFP expression, supports the notion that ECC-1 cells become infected with virus expressing T/F env.
- while numbers of T/F HIV-1 infected cells are low, they are higher than for reference env BaL, SF162, NL4-3, YU-2 and JRCSF, consistent with LucR data.
- GFP+ cells persisted in culture (beyond 14 days), and positive daughter cells were observed, suggesting expression beyond cell division from integrated DNA.
In ECC-1 cells, at the given concentrations, none of the (co-) receptor inhibitors results in a significant reduction of LucR RLU. In TZMbl control, TAK-779 results in one- to three-log reductions of LucR RLU, AMD3100 has no significant effect on R5-tropic envs, and the effect of sCD4 is env-dependent.

Polarized ECC-1 cells are CD4\textsubscript{low} and, unlike primary UEC, CCR5 \textsubscript{low/-} * → are alternative receptors used (e.g. GalCer, CCR3)?

* Asin et al., JID187 (2003)
Effect of RT and IN inhibitors on ECC-1 cell infection

- The RT and IN inhibitors, Nevirapin and Raltegavir, inhibit LucR expression.
- While reporter gene expression is generally low, it appears to be specific, and dependent on reverse transcription and integration.

* Asin et al., JID187 (2003)
Evidence for infection of primary uterine epithelial cells by HIV-1 encoding transmitted env genes

- Using 15 transmitted and 4 reference Env-IMC-LucR viruses, we found that apical exposure of polarized primary UEC to several transmitted but not reference HIV-1 reproducibly lead to reporter gene expression, indicative of infection.
- Among primary UEC from 6 different donors, we detected a wide range of LucR RLU values, suggesting tissue donor-dependent variability which might correlate with menstrual cycle stage; however, menstrual stage was not determined here.
- Results of low but reproducible LucR activity were recapitulated when polarized ECC-1 cells were exposed apically, but not basolaterally.
- Infection / LucR expression was inhibited by the RT and IN inhibitors, Nevirapin and Raltegravir.
- In an alternative approach, using GFP-reporter viruses GFP+ cells persisted in culture (beyond 14 days), and positive daughter cells were observed, suggesting integration of proviral DNA.
- That we do not observe gene expression after infection with reporter virus encoding the commonly used BaL env is in agreement with several prior reports on its lack of UEC infection. This underscores the relevance of our approach – the utilization of actual transmitted HIV-1 strains to elucidate possible targets of infection.
The UFRT is not a sterile environment, and these tissues likely become exposed to HIV-1.

To our knowledge, this is the first time HIV-1 susceptibility of UFRT cells has been addressed with *bona fide* mucosally transmitted HIV-1.

Our data suggest that uterine epithelial cells are possible targets of HIV-1 infection and transmission.

If UEC became productively infected *in vivo*, even at a low rate, and were able to support proviral gene expression over days, the proximity of other immune target cells, and the suppression of cellular immune defenses during the secretory phase, might present favorable conditions for establishment of a spreading infection - during a “Window of Vulnerability”.

Future work will further test our hypothesis and analyze other UFRT primary cell types for susceptibility to T/F HIV-1.
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Where is the route to preventing HIV-1 transmission?

Youngest participant at the March 2010 HIV Vaccine Keystone Symposium

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Effect of (co-)receptor inhibitors on ECC-1 cell infection

→ Control – inhibition of TZMbl infection

- **TZMbl** – used in standardized infection/inhibition assays; CD4+/CXCR4+/CCR5+

- In TZMbl, at the given concentrations, TAK-779 result in one- to three-log reductions of LucR RLU, AMD3100 has no significant effect on R5-tropic envs, and the effect of sCD4 is env-dependent.
Effect of RT and IN inhibitors on ECC-1 cell infection

- In ECC-1 cells, T20 + Nevirapin combination treatment (not shown) is inhibitory.

* Asin et al., JID187 (2003)
Figure 3. Analysis of T/F virus infection of MDM, using NLENG1i_Env.ecto reporter viruses
• From a viral perspective, what times during the menstrual cycle come closest to being optimal for infection?

• Within the FRT during a normal menstrual cycle, there is a period lasting 7–10 days when important components of innate, humoral, and cell-mediated immunity are suppressed by estradiol and/or progesterone, possibly enhancing the potential for viral infection. Our working hypothesis is that immunological suppression occurs in both the upper and the lower FRT as an integral part of the physiological processes that underlie successful reproduction, and that this suppression coincides with recruitment of potentially infectable cells and upregulation of coreceptors on target cells that are essential for viral uptake.

• From a viral standpoint, only the presence of antimicrobials in Fallopian tube, uterine, and endocervical secretions stands as an obstacle to successful infection during this phase.