Protección inmunitaria de las células susceptibles a la infección por VIH en el aparato reproductor femenino.

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Immune Protection of HIV susceptible cells in the Female Upper Reproductive Tract

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Schematic of the Mucosal Immune System Throughout the Human Female Reproductive Tract

10-20% of CELLS in FRT are CD45-POSITIVE CELLS
Sex Hormones Regulate Multiple Levels of Immune Protection in the Female Reproductive Tract

Effects of FRT Location, Environment and Sex Hormones:

a. Epithelial cells
b. CD8$^+$ T cells
c. CD4$^+$ T cells and macrophages
d. Hormone effects on HIV infection
e. FRT tissue environment on HIV infection
Preparation of Epithelial Cell and Stromal Fibroblast Secretions for Measuring Immune Cell Function in the FRT

Enzymatic digestion of FRT tissues to obtain epithelial & stromal cells

Epithelial Cell Sheets
- APICAL
- BASOLATERAL

Epithelial cell CM

Stromal Cell Suspension

Stromal Fibroblasts

Fibroblast CM

Menstrual Cup

Squamous Epithelial cells
Constitutive Levels of Cytokines/Chemokines/Antimicrobials in Apical Uterine Epithelial Cell Secretions

<table>
<thead>
<tr>
<th>CHEMOKINE/CYTOKINE</th>
<th>pg/ml</th>
<th>SEM ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 (CXCL8)</td>
<td>65,180</td>
<td>26,013</td>
</tr>
<tr>
<td>IL-6</td>
<td>23,400</td>
<td>5,720</td>
</tr>
<tr>
<td>SLPI</td>
<td>18,920</td>
<td>1,050</td>
</tr>
<tr>
<td>G-CSF</td>
<td>15,100</td>
<td>4,210</td>
</tr>
<tr>
<td>MCP-1 (CCL2)</td>
<td>7,800</td>
<td>890</td>
</tr>
<tr>
<td>RANTES (CCL5)</td>
<td>5,197</td>
<td>128</td>
</tr>
<tr>
<td>MIP-3a (CCL20)</td>
<td>4,461</td>
<td>130</td>
</tr>
<tr>
<td>SDF-1 (CXCL12)</td>
<td>4,237</td>
<td>248</td>
</tr>
<tr>
<td>IP-10 (CXCL10)</td>
<td>1,970</td>
<td>278</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>860</td>
<td>126</td>
</tr>
<tr>
<td>TNFα</td>
<td>235</td>
<td>79</td>
</tr>
<tr>
<td>HBD2</td>
<td>213</td>
<td>93</td>
</tr>
<tr>
<td>MIP-1b (CCL4)</td>
<td>130</td>
<td>13</td>
</tr>
</tbody>
</table>

* anti-HIV activity

Estradiol Increases SLPI Secretion and HBD2 Expression by Polarized Primary Uterine Epithelial Cells

Estradiol and Progesterone Decrease HBD2 and Elafin Secretion by Primary Vaginal Squamous Epithelial Cells

Secretions from Primary Uterine and Vaginal Epithelial Cells Inhibit X4 and R5 HIV Replication in TZM Cells

**Uterus/Fallopian Tube**

<table>
<thead>
<tr>
<th>Virus</th>
<th>UT CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>X4/T-Tropic (IIIB)</td>
<td>80%</td>
</tr>
<tr>
<td>R5/M-tropic (Bal)</td>
<td>60%</td>
</tr>
</tbody>
</table>

**Vagina**

<table>
<thead>
<tr>
<th>Relative Light Units (RLU)</th>
<th>Media</th>
<th>Bal (1)</th>
<th>Control</th>
<th>P&lt;sub&gt;4&lt;/sub&gt;</th>
<th>E&lt;sub&gt;2&lt;/sub&gt;</th>
<th>E&lt;sub&gt;2&lt;/sub&gt;/P&lt;sub&gt;4&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.0 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>5.0 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

**Pre-incubation:**
- Incubate secretion (1:10) with HIV IIIB and Bal 1h at 37°C
- Add virus + secretion to TZM cells

Wira CR et.al. Mucosal Immunology 4:335-42 (2011)
Lymphoid Aggregates in the Uterine Endometrium: B Cell Core (CD19) Surrounded by a Mantle of T Cells (CD3) Which Is Accompanied by an Outer Halo of Macrophages (CD14).

Cx/Vagina: No aggregates present.

Hormonal State Changes Cytotoxic T Cell (CTL) Activity in the Human Uterus

**Uterus:** Cyclic changes observed.


**Cx/Vagina:** No Cyclic changes observed. White et al. AJRI 37:30-38, 1997.
Experimental Design: Effect of Estradiol on HIV-infection of CD4^{+}T Cells and Macrophages

- PBMC
- CD14^{+}
- Monocytes
- Magnetic bead selection
- CD4^{+}T cells
- Activation
- IL-2+PHA
- ± E_{2}
- Activated CD4^{+}T cells
- In vitro differentiation
- Macrophages
- ± E_{2}
- HIV-infection (2h)
- PRE-infection
- POST-infection
- Estradiol (1-3 days)
- Post Estradiol (7 days)
Estradiol Reduces Susceptibility of Blood CD4$^+$ T cells and Macrophages to HIV Infection (BaL)

**CD4$^+$ T cells**

- Inf control
- pre E2
- prepost E2
- post E2

**Macrophages**

- Inf control
- pre E2
- prepost E2
- post E2

Ethinyl Estradiol (EE) has no Effect on Blood CD4⁺T Cells but Reduces HIV Infection of Macrophages (p24)

CD4⁺T cells

Macrophages

Estradiol Reduces Susceptibility of Blood and FRT CD4⁺ T Cells to HIV Infection


Rodriguez-Garcia et al. Submitted.
Conclusions

1. E₂ and P regulate antimicrobial secretion by epithelial cells (UT vs. VG), some of which have anti-HIV activity.

2. Sex hormones control CTL activity in FRT tissues. Varies with site and stage of menstrual cycle.

3. CD4⁺ and CD8⁺ T cell numbers vary with the FRT site examined (EM< CX and ECX).

4. CD4⁺ Th17 Cells in the FRT vary with the site in the FRT analyzed and are different from blood (ROR-γ, IL-17 CCL20).

5. FRT CD4⁺ T Cell susceptibility to HIV Infection is low in UT relative to CV and ECX.
Conclusions

6. Estradiol reduces the susceptibility of blood CD4\(^+\) T cells and Macrophages to HIV Infection (BaL).

7. E\(_2\) but not EE inhibits HIV infection of CD4\(^+\) T cells, both inhibit Macrophage infection.

8. Estradiol reduces HIV infection of CD4\(^+\) T Cells from blood and from the FRT (CX and ECX).

9. Epithelial cells and fibroblast secretions (CM) protect against HIV infection in the FRT.

Overall, the FRT provides protection to HIV-susceptible cells at several levels, including antimicrobial secretion and CTL activity as well as direct and indirect effects of sex hormones on CD4\(^+\) T cells and macrophages.
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