Immunological Changes in the FRT During the Menstrual Cycle

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Anatomy and Cell Distribution

- Dendritic Cell
- Stromal Cell
- Macrophage
- B cell
- T cell
- NK cell

Upper tract

Innate immunity
Humoral immunity
Cellular immunity

Lower tract
Hormone Regulation of Immune Function in the Human Female Reproductive Tract (Uterus and Vagina)

Enzymatic digestion of FRT tissues to obtain epithelial & stromal cells

Stromal Cell (Fibroblasts and Immune Cells)

Epithelial Cell Sheets

APICAL

BASOLATERAL

FRT Cells

Vaginal Cells

Estradiol (0.1-5x10^{-8}M)

CD4+ T Cells

96-well Culture Plates

Magnetic bead selection
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 Infection of TZM Cells

**Uterus/Fallopian Tube**

![Bar chart showing % of Virus Control for X4/T-Tropic (IIIB) and R5/M-tropic (Bal) with UT CM and Virus comparisons.]

**Vagina**

![Bar chart showing Relative Light Units (RLU) for Media, Bal (I), Control, P4, E2, and E2/P4 conditions with Vaginal CM.]

- **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)
Unique mRNA Expression of CD4+Th17 Cell Markers in the Upper and Lower FRT

Rodriguez-Garcia et al; Mucosal Immunology. Online publication 23 April 2014. doi:10.1038/mi.2014.26
Differential Susceptibility of CD4+ T Cells from the FRT to HIV Infection

Intracellular p24

Rodriguez-Garcia et al; Mucosal Immunology. Online publication 23 April 2014. doi:10.1038/mi.2014.26
Estradiol Reduces Susceptibility of Blood CD4\(^+\) T Cells to HIV Infection

Comparison of TFV-DP levels in epithelial cells, fibroblasts, CD14+ cells and CD4+ T cells from FRT tissues.

Stimulatory Effect of E$_2$ and/or P4 on TFV-DP Levels in Epithelial Cells from Endometrium, Endocervix/Ectocervix

Inhibitory Effect of Estradiol and/or Progesterone on TFV-DP Levels in CD4$^+$ T Cells from FRT

Day 6 values

- Protein: 1280 ug/ml
- IL-1a: 93 pg/ml
- IL-6: 87 pg/ml
- IL-8: 533 pg/ml
- IL-1Ra: 30 ng/ml
- TGF-beta: 1408 pg/ml
- SLPI: 142 ng/ml
- HBD2: 1608 pg/ml
- HNP1-3: 131 ng/ml
- Lactoferrin: 378 ng/ml
- IgG: 4045 ng/ml
- IgA: 552 ng/ml

Profile of Vaginal Secretions Recovered by Menstrual Cup from Individual Volunteers

Patel et al AJRI 2014 In Press
Individual Profiles of Anti-HIV activity in Vaginal Secretions

A

BaL

Relative Light Units (RLU)

Consecutive Menstrual Cycle Stage

B

IIIB

Relative Light Units (RLU)

Consecutive Menstrual Cycle Stage

C

CH077.t

Relative Light Units (RLU)

Consecutive Menstrual Cycle Stage

D

CH058.c

Relative Light Units (RLU)

Consecutive Menstrual Cycle Stage

Patel et al AJRI 2014 In Press
Cytokine and Chemokine Concentrations in Lower FRT Secretions from Multiple Studies

Adapted from (Hein et al., 2002, Keller et al., 2007, Shust et al., 2010, Keller et al., 2012b, Keller et al., 2012a, Keller et al., 2011, Keller et al., 2010, Madan et al., 2012, Levinson et al., 2012, Levinson et al., 2009, Valore et al., 2002, Dezzutti et al., 2011, Kyongo et al., 2012, Anderson et al., 2012, Ghosh et al., 2010b, Walter et al., 2011).
Comparison of Antimicrobial Concentrations in Lower FRT Secretions from Multiple Studies

Adapted from (Hein et al., 2002, Keller et al., 2007, Shust et al., 2010, Keller et al., 2012b, Keller et al., 2012a, Keller et al., 2011, Keller et al., 2010, Madan et al., 2012, Levinson et al., 2012, Levinson et al., 2009, Valore et al., 2002, Dezzutti et al., 2011, Kyongo et al., 2012, Anderson et al., 2012, Ghosh et al., 2010b, Walter et al., 2011).
Differences in Cytokine and Chemokine Concentration Between Endometrial Secretions and Cervical Mucus


* = significantly different. Data derived from (Boomsma et al., 2009a, Boomsma et al., 2010 Boomsma et al., 2009b).
Epithelium Immaturity as a Risk Factor for HIV Acquisition: Colposcopy Quantitative Measurement of Cervical Immaturity

Cervical Transition Zone

Immature = 67% of total space.

Mature = 95% of total space.

<table>
<thead>
<tr>
<th></th>
<th>Immature epithelium (n=16)</th>
<th>Mature epithelium (n=16)</th>
<th>p-value $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>19.5 (2.5)</td>
<td>18.8 (1.8)</td>
<td>0.39</td>
</tr>
<tr>
<td>Age of menarche</td>
<td>12.3 (1.8)</td>
<td>12.5 (1.8)</td>
<td>0.72</td>
</tr>
<tr>
<td>Years of sexual activity</td>
<td>3.5 (2.7)</td>
<td>3.3 (1.8)</td>
<td>0.78</td>
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<tr>
<td>Days since last menses</td>
<td>19 (17, 35)</td>
<td>17 (10, 23)</td>
<td>0.26</td>
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<tr>
<td>Number of lifetime sexual partners</td>
<td>4 (2.9)</td>
<td>3 (3.4)</td>
<td>0.70</td>
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</tbody>
</table>

Protein Levels of Cervical Vaginal Cytokine/Chemokines in Healthy Young Women with Immature and Mature Cervical Epithelium

<table>
<thead>
<tr>
<th>Cytokine$^a$</th>
<th>Immature epithelium (n=16)</th>
<th>Mature epithelium (n=16)</th>
<th>Box Cox regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (pg/ml)</td>
<td>Interquartiles (pg/ml)</td>
<td>Median (pg/ml)</td>
</tr>
<tr>
<td>IL-1α</td>
<td>395.89</td>
<td>89.65</td>
<td>894.96</td>
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<tr>
<td>IL-1β</td>
<td>54.11</td>
<td>11.7</td>
<td>261.43</td>
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<tr>
<td>IL-6</td>
<td>50.01</td>
<td>21.07</td>
<td>94.01</td>
</tr>
<tr>
<td>IL-8</td>
<td>3139.1</td>
<td>401.8</td>
<td>7557.2</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>24.39</td>
<td>10.02</td>
<td>109.18</td>
</tr>
<tr>
<td>RANTES</td>
<td>2.55</td>
<td>2.00</td>
<td>15.42</td>
</tr>
<tr>
<td>TNF</td>
<td>0.67</td>
<td>0.63</td>
<td>0.73</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.69</td>
<td>0.50</td>
<td>1.54</td>
</tr>
<tr>
<td>IL-12</td>
<td>0.9</td>
<td>0.81</td>
<td>1.01</td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.30</td>
<td>0.30</td>
<td>0.66</td>
</tr>
</tbody>
</table>

<LLD, below the lower limit of detection; IL, interkeukin; MIP, macrophage inflammatory protein; RANTES, regulated upon activation.


2. Tissue environment influences HIV susceptibility of CD4+ T cells in FRT.

3. Secretions in lower FRT are composed of vaginal transudate, vaginal EC secretions, cervical mucus/secretions and uterine fluid.


6. Quantity of antimicrobials/cytokines in the secretions (ELISA) does not provide a complete picture of their biological activity: ie. Cathepsin D, under hormonal control, inhibits the function of CCL20.

7. Some proteins inhibit and enhance HIV infection in vitro (concentration dependent): Example - RANTES, IL-8, and SDF-1a inhibit; RANTES increases HIV replication in monocytes/macrophages. IL-8 stimulates HIV replication in T cells and macrophages.

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