Female genital epithelial cells from HIV-exposed seronegative commercial sex workers express a discrete cytokine/chemokines profile upon toll-like receptor activation

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Background

- Women are disproportionately susceptible to sexually transmitted viral infections. Recent UNAIDS estimates indicate that globally, 30-40% of annual HIV infections occur through heterosexual transmission in the female genital tract (FGT). In sub-Saharan Africa, currently, 57% of all people infected with HIV are female. Therefore, measures to prevent heterosexual transmission of HIV-1 are urgently needed to restrain growth of the acquired immunodeficiency syndrome.
- Innate immune mechanisms in the FGT may play a central role in acute HIV infection.
- Epithelial cells that line the FGT play a key role in forming a primary physical barrier against HIV and other sexually transmitted viruses.
- Besides providing a physical barrier, FGT epithelial cells can directly recognize and respond to pathogens, including HIV-1.
- Proinflammatory cytokines and chemokines and other innate biological factors produced by FGT epithelia can either serve to prevent or facilitate HIV-1 infection.
- Lower FGT is lined with two distinctive epithelial cell types. The epithelial lining of vagina and ectocervix consists of multiple layers of stratified squamous epithelial cells, and the endocervical epithelium consists of a single layer of columnar-type cells.
- In order to develop effective mucosal preventive measures against HIV-1 infection, it is crucial to understand how FGT epithelial cells respond to pathogen-associated molecular patterns derived from HIV-1, other sexually transmitted pathogens and/or vaginal commensal microbiota.
- Immune cells localized by TLR mediated activation of female genital tract epithelial cells can be a critical determinant of HIV-1 resistance or susceptibility.
- HIV-1-exposed seronegative (HESN) women have been shown to have a distinct pattern of cytokines and chemokines as measured in CVL samples. In this study, we investigated the role of TLR signaling in determining the local mucosal cytokine/chemokines milieu in genital epithelial cells from HESN, HIV-1 infected and HIV-1 negative Kenyan commercial sex workers (CSWs).

Methods

- Total RNA was extracted using RNeasy Plus mini kit (QIAGEN) and was reverse transcribed (RT; QIAGEN). Resulting cDNA was evaluated in real-time quantitative PCR using SYBR Green qPCR Master Mix (QIAGEN) with primer sets specific to each TLR, RLR and NLR transcripts and 18s RNA with proper RT controls.
- Endocervical cytobrush samples were obtained from Pumwani CSWs cohort. Cervical epithelial cells (CECs) were purified through a series of nylon membrane filtrations. Purity and viability of CECs was assessed by cytokeratin expression and MTS assay, respectively.
- Cytokine and Chemokine levels in 24 h culture (with or without 25 ug/ml Poly(I:C), 1ug/ml Pam3CSK4, 1ug/ml LPS, 100 ng/ml Flagellin and 100ng/ml FSL-1 combined) supernatants were determined using the Milliplex MAP multiplex Human Cytokine/Chemokine kit (Millipore) and analyzed on the BioPlex-200 Bio-Rad according to the manufacturer’s protocol.

Results

Toll-like receptor expression in FGT

- Toll-like receptor gene expression in endocervical epithelial cells

IL-8 production upon TLR3 or combined TLR1/2, 4, 5, and 6 stimulation

IP-10 production upon TLR3 or combined TLR1/2, 4, 5, and 6 stimulation

Summary of Results and Conclusions

- TLR1, TLR2, TLR3, TLR5, and TLR9 are all expressed in vaginal, ectocervical and endocervical epithelial cells. However, expression varies greatly between cells obtained from different portions of FGT with the exception of TLR6.
- TLR4 is only expressed in endocervical epithelial cells.
- TLR7, TLR8 and TLR10 are not expressed in epithelial cells derived from any of the three lower FGT locations.
- Expression of Cytosolic DNA helicases (RIG-I and MDA-5) and CATERPILLERS (NOD1 and NOD2) varies between vaginal, ectocervical and endocervical epithelial cells.
- Generally, epithelial cells derived from endocervical area respond more intensively to PRR stimulation.
- CEcs from HESN subjects seem to have a better control of mucosal inflammation.

Acknowledgements

Table 1: Toll-like receptors gene expression in normal or HIV-1 infected female genital epithelial cells. Cytokine/chemokines measured in 24h culture supernatants via Milliplex MAP array.

Figure 1: Toll-like receptor gene expression normalized to 18s RNA expression via real-time PCR.

Figure 2: Pattern recognition receptor gene expression normalized to 18s RNA expression via real-time PCR.

Figure 3: Endocervical epithelial cells were cultured with or without 25 ug/ml Poly(I:C), or 1ug/ml Pam3CSK4, 1ug/ml LPS, 100 ng/ml Flagellin and 100ng/ml FSL-1 combined. Cytokine/chemokines measured in 24h culture supernatants via Milliplex MAP array.

Figure 4: Toll-like receptor gene expression normalized to 18s RNA expression via real-time PCR.

Figure 5: Endocervical epithelial cells were cultured with or without 25 ug/ml Poly(I:C), or 1ug/ml Pam3CSK4, 1ug/ml LPS, 100 ng/ml Flagellin and 100ng/ml FSL-1 combined. Cytokine/chemokines measured in 24h culture supernatants via Milliplex MAP array.

Figure 6: Endocervical epithelial cells were cultured with or without 25 ug/ml Poly(I:C), or 1ug/ml Pam3CSK4, 1ug/ml LPS, 100 ng/ml Flagellin and 100ng/ml FSL-1 combined. Cytokine/chemokines measured in 24h culture supernatants via Milliplex MAP array.

Figure 7: Endocervical epithelial cells were cultured with or without 25 ug/ml Poly(I:C), or 1ug/ml Pam3CSK4, 1ug/ml LPS, 100 ng/ml Flagellin and 100ng/ml FSL-1 combined. Cytokine/chemokines measured in 24h culture supernatants via Milliplex MAP array.