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, and 76% of young people (aged 15–24) living with HIV are female. Therefore measures to prevent hetero-sexual mucosal 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 microbes.
- Immune locale orchestrated 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

- The Pumwani Sex Worker Cohort was established in 1985 for a study on the immunobiology and epidemiology of sexually transmitted infections. Over 3,000 women have been enrolled in this open cohort. In all, 22 HESN CSWs, 24 HIV-1-negative CSWs, and 23 HIV-1-infected CSWs were included in this study. HESN CSWs were defined as active CSWs who have remained HIV-1 seronegative and HIV-1 negative by PCR for at least 7 years of follow-up. HIV-1-negative CSWs are women newly enrolled in the cohort (for <3 years) and HIV-1 seronegative.
- Endocervical End1/E6E7, ectocervical Ect1/E6E7 and vaginal Vk2/E6E7 epithelial cell lines (ATCC), were maintained in keratinocyte serum-free medium supplemented with bovine pituitary extract, epidermal growth factor, 100 U/ml penicillin, 100 mg/ml streptomycin, and CaCl2.

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 for each TLR, RLR and NLR transcripts and *I8S rRNA* with proper RT controls.
- Endoervical cytobrsuh 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), or 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 I (Millipore) and analyzed on the BioPlex-200 (Bio-Rad) according to the manufacturer's protocol.

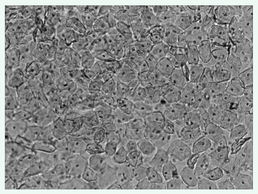

***Figure 1:** Cultured primary FGT epithelial cells isolated from endocervical cytobrush samples.*

Results

Toll-like receptor expression in FGT

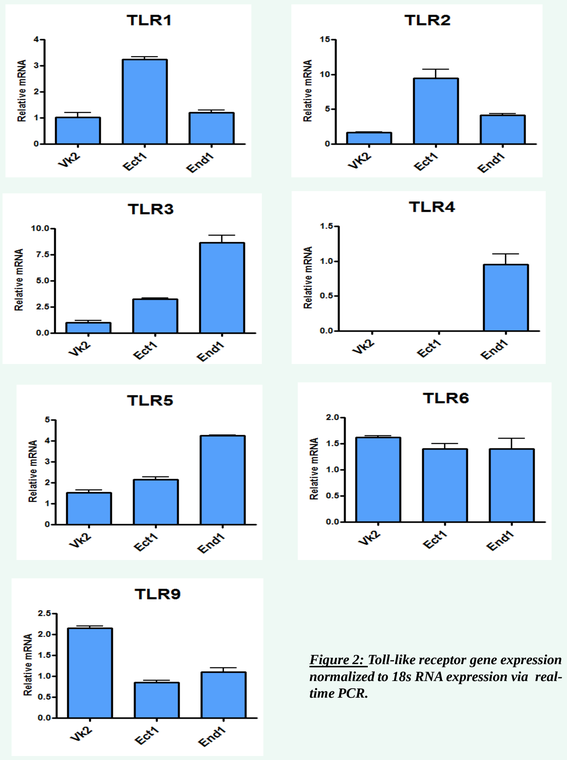

***Figure 2:** Toll-like receptor gene expression normalized to 18s RNA expression via real-time PCR.*

Cytoplasmic pattern recognition receptors expression in FGT

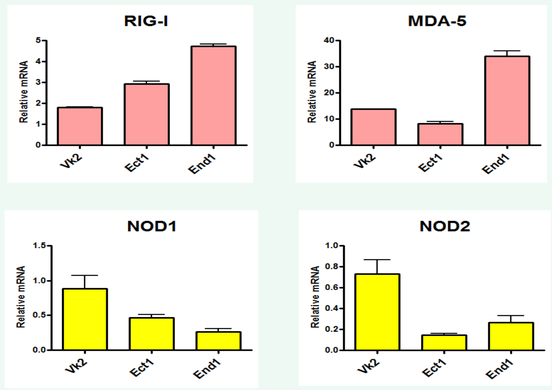

***Figure 3:** RIG-1, MDA-5, NOD1 and NOD 2 gene expression normalized to 18s RNA expression via real- time PCR*

Pattern recognition receptor expression in endocervical epithelial cells

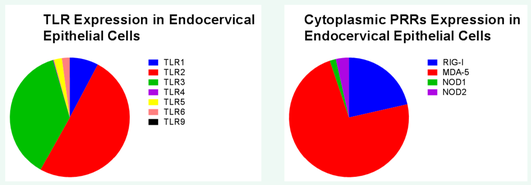

***Figure 4:** Pattern recognition receptor gene expression normalized to 18s RNA expression via real- time PCR*

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

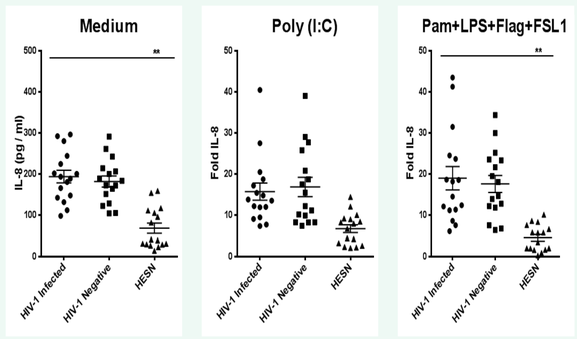

***Figure 5:** CECs were cultured with or without 25 ug/ml Poly(I:C), or 1ug/ml Pam3CSK4, 1ug/ml LPS, 100 ng/ml Flagellin and 100ng/ml FSL-1 combined) . Cytokines/chemokines measured in 24h culture supernatants via Milliplex MAP assay.*

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

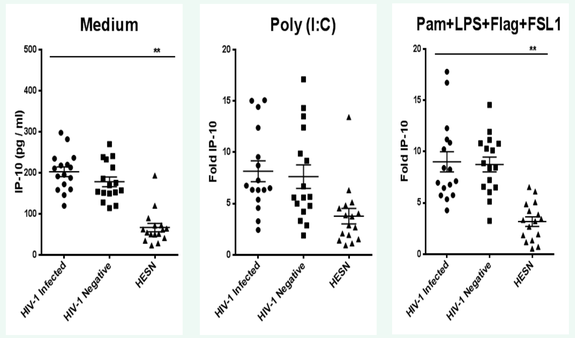

***Figure 6:** CECs were cultured with or without 25 ug/ml Poly(I:C), or 1ug/ml Pam3CSK4, 1ug/ml LPS, 100 ng/ml Flagellin and 100ng/ml FSL-1 combined) . Cytokines/chemokines measured in 24h culture supernatants via Milliplex MAP assay.*

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 cell 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 Cytoplasmic RNA 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 produce significantly lower levels of basal as well as TLR mediated IL-8 and IP-10.
- In conclusion, we show that HIV-1-resistant women have a distinct pattern of mucosal epithelial derived chemokine/cytokine expression that contributes to the immune quiescence phenotype observed in the Pumwani HESN cohort. HESN subjects showed lower levels of basal and TLR mediated IL-8 and IP-10 that may be critical at reducing trafficking to this crucial mucosal site.
- HESN subjects seem to have a better control of mucosal inflammation.

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