Virology 493 (2016) 247–254

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Female genital tract inflammation, HIV co-infection and persistent mucosal Human Papillomavirus (HPV) infections

Jean-Mari Kriek [a,1], Shameem Z. Jaumdally [a], Lindi Masson [a], Francesca Little [b], Zizipho Mbulawa [a,c], Pamela P. Gumbi [a], Shaun L. Barnabas [a,d], Jennifer Moodley [e,f,g], Lynette Denny [e,h], David Coetzee [i], Anna-Lise Williamson [a,e,j], Jo-Ann S. Passmore [a,e,j,*]

[a] Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
[b] Department of Statistical Science, University of Cape Town, Cape Town, South Africa
[c] Center for HIV and STIs National Institute for Communicable Disease, National Health Laboratory Service, South Africa
[d] Desmond Tutu HIV Foundation, University of Cape Town, Cape Town, South Africa
[e] UCT/MRC Clinical Gynaecological Cancer Research Centre, University of Cape Town, Cape Town, South Africa
[f] Cancer Research Initiative, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
[g] Women's Health Research Unit, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa
[h] Department of Obstetrics and Gynaecology, University of Cape Town/Groote Schuur Hospital, Cape Town, South Africa
[i] School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa
[j] National Health Laboratory Service, South Africa

ARTICLE INFO

Article history:
Received 18 January 2016
Returned to author for revisions
24 March 2016
Accepted 28 March 2016

Keywords:
Mucosal
HPV
Persistence
HIV
Inflammation
Cytokines

ABSTRACT

Background: Persistent genital infections with high-risk HPV types increase risk of cervical disease and cancer. Since genital inflammation increases HIV acquisition risk and cancer progression, we evaluated whether HPV infection induces cytokine expression in the reproductive tract.
Methods: Genital cytokines concentrations were measured in 93 HIV-infected and 72 uninfected women. HPV typing was done by Roche Linear array. Persistence and clearance of HPV were evaluated using longitudinal data.
Results: Infection with HPV did not influence genital cytokine concentrations. In contrast, HIV-infected women had higher IL-1α, IL-6, IL-8, IP-10, MCP-1 and G-CSF concentrations compared to HIV-uninfected women, and HPV-infections that were more prevalent, persistent and multi-type.
Conclusion: HPV did not influence inflammatory cytokine levels in the genital tract, although immune suppression may favor persistence.

Importance

Vaginal infections with certain human papillomavirus (HPV), if not treated or cleared, can increase women's risk of developing cervical cancer. HIV infection can exacerbate HPV infections. We explored whether host immunity influenced the duration of HPV infections and whether host immunity is altered by an infection. In HIV negative women, we found that HPV infections do not change levels of vaginal inflammation, but that immune regulating responses were lower in those who failed to clear their infections. HIV-positive women were more likely to have an HPV infection and significantly higher inflammatory responses, potentially increasing their risk for cervical cancer.

1. Introduction

Human Papillomaviruses (HPVs) are transmitted during sexual intercourse. Approximately 80% of sexually active individuals acquire genital HPV during their lifetime Einstein et al. (2009). Although 90% of infected immunocompetent women clear infections within 24 months (Wentzensen et al., 2009), those in whom infection persists have a significantly higher risk of cervical cancer (WHO/ICO, 2010). Mucosal HPV types are categorized by their risk for causing cervical cancer into low- or high-risk types. Low-risk HPV is a common cause of genital warts, while most cervical and anogenital cancers are caused by persistent high-risk HPV (Castle et al., 2001; Brown et al., 2009). High-risk HPV-16 and -18 account

* Corresponding author at: Institute of Infectious Disease and Molecular Medicine, Division of Medical Virology, Faculty of Health Sciences, University of Cape Town, Anzio Road, Observatory 7925, Cape Town, South Africa.
E-mail address: Jo-ann.Passmore@uct.ac.za (J.-A. Passmore).
[1] Jean-Mari Kriek and Shameem Jaumdally contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.virol.2016.03.022
0042-6822/

for > 70% of cervical cancer cases, with HPV-31, -33, -45, -52, and -58 accounting for 20% (Clifford et al., 2006; Awolude et al., 2013).

A link between HPV infection and increased HIV risk has been reported (Houlihan et al., 2012), with HPV clearance, not persistence, correlating with increased HIV risk (Smith-McCune et al., 2010). The mechanism, however, has not yet been determined. Many studies report that, relative to HIV-negative women, HIV-infected women are at higher risk for cervical disease progression partly because they are more likely to have HPV infections (Mbulawa et al., 2012).

We hypothesized that HPV infections may alter the cytokine milieu in the genital tract and that a cytokine signature may signal clearance or persistence. This study investigated whether HPV infection was associated with increased concentrations of genital cytokines, and if specific cytokines predicted the persistence or clearance of HPV infections over six months, in HIV-uninfected and HIV-infected women.

2. Methods

2.1. Study participants

One hundred and sixty-five pre-menopausal, heterosexual women were recruited from the Empilisweni Center, Gugulethu, Cape Town (Gumbi et al., 2008; Mbulawa et al., 2009). Exclusion criteria included menstruation, hysterectomy, and a symptomatic STI. Eighty-five women returned 6 months later for follow-up. The University of Cape Town Faculty of Health Sciences Research Ethics Committee approved the study.

2.2. Collection of genital tract specimens

At baseline, two endocervical cytobrushes were collected using Digene cervical samplers, as previously described (Passmore et al., 2002), for cytokine studies, and HPV genotyping. Cytobrushes were transported in RPMI-1640 medium (GIBCO®) supplemented with 10% FCS, 50 µl Penicillin, 50 mg/ml Streptomycin, 0.8 mg/ml Fungin and 50 mg/ml L-glutamine, flushed, and centrifuged at 1000*g* for 10 min (Mbulawa et al., 2010). Cervical cytobrush supernatants were used for genital cytokine concentration analysis as representative samples of the transformation zone where HPV-infections occur most frequently (Moscicki et al., 2006). At visit 2, one endocervical cytobrush was collected for HPV typing only.

2.3. HPV genotyping and definitions of incident, persistence or clearance of HPV

DNA extracted from cervical cytobrush samples (MagNa Pure Compact Nucleic Acid Isolation Kit Roche Diagnostics, Germany) was used for HPV genotyping with the Roche Linear Array HPV Genotyping assays which detects 37 different HPV genotypes and sample adequacy with β-globin. High-risk HPV types included HPV-16, -18, -26, -31, -33,-35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73 and -82, while low-risk HPV types included HPV-6, -11, -40, -42, -54, -55, -61, -62, -64, -67, -69, -70, -71,-72, -81, -83, -84, -89 (HPV-CP6108) and IS39 (Mbulawa et al., 2009). The α-9 HPV species included HPV-16, -31, -33, -35, -52, -58 and -67 (Mbulawa et al., 2014).

Women who were HPV-negative at both visits were the control group (HPV-negative); those HPV-negative at visit 1 and acquired any HPV at visit 2 were classified as having incident infections; those with any HPV infection at visit 1 that was also detected at visit 2 were classified as persistently infected; and women with a specific HPV type at visit 1 that was absent at visit 2 were classified as having cleared their infections. The stricter definition of clearance, clearing all types by visit 2, was not applied as only 8/85 women met this criterion. Since we evaluated changes in HPV prevalence during a 6-month period, it is likely that clearance or persistence may be differently classified over a longer period.

2.4. Screening for sexually transmitted infections (STIs)

Genital samples were screened for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* by PCR at the STI Surveillance Laboratory, National Health Laboratory Services (Lewis et al., 2013).

2.5. Measurement of HIV viral loads

HIV viral loads were determined in plasma using the Nuclisens Easyq HIV 1 Version 1.2 (Gumbi et al., 2008), which has a lower detection limit of 50 HIV RNA copies/ml.

2.6. Measurement of genital tract cytokines

Eight cytokines were measured in cervical cytobrush supernatants using Human Cytokine Milliplex kits (Millipore, USA): Interleukin (IL)-8, IL-6, IL-10, interferon-γ induced protein (IP)-10, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1β, IL-1α, and granulocyte colony-stimulating factor (G-CSF). Cytokine selection was based on their association with STIs, however since the concentrations of TNFa and IFNa are very low in cervical cytobrush supernatant, we did not include these cytokines in our panel (Masson et al., 2014) and their inter-assay reliability (Spearman Rank inter-assay correlation coefficients > 0.8, Table 2). The kit sensitivity ranged from 0.3 to 3.9 pg/ml. Cytokine data was collected using a Bio-Plex Suspension Array Reader (Bio-Rad Laboratories Inc., USA). Concentrations below the limits of detection were reported as the midpoint between the lowest concentration and zero (Masson et al., 2014).

2.7. Statistical analysis

Statistical analysis was conducted using GraphPad Prism version 5.0 (USA) and STATA™ version 11 (StataCorp, USA). Fisher's exact test was used to compare proportions, Mann Whitney *U* test was used for comparisons and Spearman Rank test for correlations. Cytokine concentrations were $\log_{10}$-transformed to improve distribution. Logistic regression was used to examine the relationships between $\log_{10}$-transformed cytokine concentrations and high-risk, low-risk or multiple HPV infections, while adjusting for other STIs. The relationships between cytokine concentrations and HPV clearance, persistence or incidence, following adjustment for HIV status and other STIs, were assessed using linear regression. Biplots were used to visualize distances in cytokine profiles between high-risk or low-risk HPV infections (relative to HPV-negative women) in a multidimensional space, as well as variances and correlations between cytokines. P-values were adjusted for multiple comparisons using a false discovery rate (FDR) step down procedure and adjusted *p*-values of < 0.05 were considered significant.

3. Results

One hundred and sixty five South African women were recruited to investigate the relationship between cytokine levels in the lower reproductive tract and persistence or clearance of HPV-infection (Table 1). Of these women, 72/165 (44%) were HIV-negative and 93/165 (56%) HIV-positive. The median age was 37 years (IQR 28–44 years). HIV-negative women were sexually active for 19 years (IQR 10–26 years), with a median of three lifetime