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Impact of asymptomatic Herpes simplex virus-2 infection on T cell function in the foreskin of men from Rakai, Uganda

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Conflicts of Interest

No author has any financial or personal relationship posing a conflict of interest in relation to this study.

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SUMMARY

Herpes simplex type 2 (HSV-2) increases the risk of HIV acquisition in men and overall CD4 T cell density in the foreskin. Using tissues obtained during routine male circumcision, we examined the impact of HSV-2 on the function and phenotype of foreskin T cells in Ugandan men. HSV-2 infection was predominantly associated with a compartmentalized increase in CCR5 expression by foreskin CD4 T cells, which may contribute to HIV susceptibility.

Keywords

HSV-2; HIV; foreskin; T cells

Susceptibility to HIV is heterogeneous [1], and chronic infection by Herpes simplex virus type 2 (HSV-2) has been associated with an approximately three-fold increase in HIV acquisition by both men and women [2]. However, most HSV-2 is asymptomatic and suppressive acyclovir in HSV-2 seropositive individuals did not reduce HIV acquisition despite reductions in clinical ulceration [3], suggesting that increased HIV susceptibility is not solely due to compromised epithelial integrity. A recent study has shown the density of CD4 T cells expressing the HIV co-receptor CCR5 is increased at the site of herpetic ulcers, even after 20 weeks of acyclovir treatment with no recurrence of ulceration and no detectable HSV DNA [4]. Additionally, a higher proportion of CD4 T cells in the cervix of HSV-2 seropositive women co-express CCR5 [5], even in the absence of prior symptomatic herpes or asymptomatic local viral reactivation [6], suggesting that changes in the cervical immune microenvironment may increase a woman's HIV susceptibility during asymptomatic HSV-2 infection.

Less is known about the immune correlates of HIV susceptibility in HSV-2⁺ men. The rollout of male circumcision (MC) in sub-Saharan Africa as an HIV prevention strategy has increased opportunities to address this gap. Studies from Rakai, Uganda demonstrated an increased density of foreskin CD4 T cells in HSV-2-infected men [7], but the functional characteristics of mucosal CD4 T cells may also be an important determinant of HIV susceptibility [8]. We recently established techniques to isolate viable T cells from preputial tissues [9], and demonstrated that, compared to the blood, the foreskin manifests a pro-inflammatory immune microenvironment, with high production of TNF α and IFN γ by CD8 T cells and high expression of CCR5 by CD4 T cells. We also found the foreskin to contain a more Th17 cells, which are important in mucosal defense against bacterial and fungal infections but are highly susceptible to HIV infection [10–12] and promote a pro-inflammatory immune environment. However, there was no corresponding increase in immunomodulatory T-regulatory cells (Tregs), which decrease local immune activation and may reduce HIV susceptibility *in vivo* [13].

Based on these findings, we hypothesized that asymptomatic HSV-2 infection would be associated with increased levels of CCR5 expression, enhanced inflammatory cytokine production, and more Th17 cells within the foreskin.

Participants were men from the established Rakai Community Cohort Study, Uganda [14] who had elected to undergo adult circumcision as a service at the Rakai Health Sciences Program. Foreskins and blood were obtained from 87 HIV-negative men, 39 of whom were found to be positive for HSV-2 by serology. All participants provided written informed consent, and ethical approval was obtained from the research ethics boards of all collaborating institutions. HIV testing was performed with two ELISAs and discordant results were confirmed by Western blot, as previously described [9]. HSV-2 serology was performed by ELISA (Herpes Simplex Type 2 IgG ELISA, Kalon Biological Ltd., Guildford, UK), as previously validated in Rakai [15]. Laboratory testing for other genital infections was not performed.

Cell isolation and immune assays were performed by personnel blinded to HSV-2 serostatus as previously described [9]. Briefly, fresh preputial tissue was disrupted with mechanical and enzymatic digestion and the resulting cell suspension was filtered to remove any undigested tissue. PBMC and foreskin cells were either stimulated with 1ng/ml phorbol-12-myristate-13-acetate (PMA) and 1µg/ml ionomycin or with vehicle (0.1% DMSO, all from Sigma; St. Louis, MO, USA). Samples were then stained for surface antigens with combinations of antibodies for CD3, CD4, CD8, CCR5, and CD25 (all BD Biosciences; Franklin Lakes, NJ USA). Samples necessitating intracellular staining were permeabilized and stained with combinations of antibodies for TNFα, IFNγ (BD Biosciences), IL17a, IL22, and FoxP3 (eBiosciences; San Diego, CA, USA). Samples were acquired using a FACSCalibur flow cytometer (BD Systems) and data analysis performed using FlowJo software (v.9.3, Treestar; Ashland, OR, USA). T cell populations were compared between HSV-2-positive and -negative individuals by Mann Whitney *U* Test. Statistical analyses used SPSS (v.19.0, IBM; New York, NY, USA).

All participants were free of symptoms or signs of genital infections (ulceration, dysuria or urethral discharge) at the time of surgery; one HSV-2 seropositive participant reported mild balanitis that was not apparent on physical exam. HSV-2 seropositive participants were older (median 36 vs. 32 years; Pearson $p=0.013$) and were more likely to have more than one sex partner over the past year (38.5% vs. 16.7%; $p=0.022$), although condom use did not vary by group (data not shown). The proportion of CD3⁺ cells expressing CD4 was non-significantly higher in both the blood (65.2 vs. 61.3%, $p = 0.172$) and the foreskin (53.1 vs. 49.3%, $p = 0.096$) of HSV-2⁺ individuals, and foreskin T cell density was not assessed. Since sexual HIV-infection occurs almost exclusively with viral strains using CCR5 as a co-receptor, expression of CCR5 on these CD4 T cells was also measured. A significantly higher proportion of foreskin CD4 T cells from HSV-2⁺ men co-expressed CCR5 (45.6 vs. 37.7%, $p = 0.024$; Figure 1a), and this association remained after controlling for age ($p=0.017$) and number of sex partners ($p=0.009$). CCR5⁺ expression did not vary by HSV-2 status in the blood compartment ($p = 0.582$).

However, there were no associations between HSV-2 infection and the frequency of CD4⁺ Th17 and T regulatory cells (Tregs) in the foreskin (foreskin: Th17: 6.1 vs. 6.2%; Treg: 3.4 vs. 3.3%; Figure 1b). Likewise, HSV-2 infection was not associated with differences in the capacity of foreskin or blood CD8⁺ T cells to produce the pro-inflammatory cytokines TNFα or IFNγ (foreskin: TNFα: 39.6 vs. 37.5%; IFNγ: 44.9 vs. 41.1%; Figure 1c).

The foreskin is the site of most HIV acquisition in uncircumcised men, in part because (compared to blood) it constitutes a pro-inflammatory mucosal immune environment. Specifically, the foreskin demonstrates higher CD4 T cell expression of the HIV co-receptor CCR5, Th17 cell frequency and CD8 T cell production of pro-inflammatory cytokines [9]. While we hypothesized that increased HIV susceptibility in HSV-2-infected men might relate to accentuation of each of these factors, we found that the major immune association of asymptomatic HSV-2 infection in the foreskin was an increase in the expression of CCR5 by CD4 T cells. Therefore, HSV-2 is not only associated with an increased density of CD4 T cells in the foreskin, as we have shown previously [7], but these CD4 T cells also express higher levels of CCR5. This compartmentalized increase of CCR5 expression on foreskin CD4 T cells may contribute to HSV-2-associated increases in HIV susceptibility.

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Figure 1. Immune associations of HSV-2 infection in the foreskin

Impact of HSV-2 infection status on: the proportion of CD4 T cells from the blood and foreskin expressing the HIV co-receptor CCR5 (B); the proportion of CD4 T cells from the blood and foreskin that are Th17 cells (producing IL-17a upon stimulation) or Tregs (co-expressing CD25 and FoxP3; (C); and on the capacity of CD8 T cells to produce the pro-inflammatory cytokines IFN γ and TNF α upon stimulation (D). Representative flow plot presented in (A). Statistical comparisons were performed using the Mann-Whitney *U* Test.