Genital Inflammation and HIV/HSV-2 Shedding Post-Male Circumcision in Rakai, Uganda

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Abstract #987
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Background

Medical male circumcision (MC) decreases heterosexual acquisition of HIV and herpes simplex virus type 2 (HSV-2).[1]

Penile HIV shedding increases in the first 1-3 weeks following MC in ART-naïve men,[2] and penile HSV-2 shedding increases in the first 1-2 weeks post-MC in HIV/HSV-2 co-infected men.[3]

This study aimed to characterize the pro-inflammatory response associated with penile HIV and HSV-2 shedding from MC wounds in HIV positive Ugandan men.

Methodology

Study Design

A prospective study of HIV-infected men undergoing MC was conducted in Rakai, Uganda. Non-traumatic penile lavage samples using PBS were collected at the coronal sulcus prior to MC, and weekly post-MC for 4 weeks.

Laboratory Testing

HIV shedding status (HIV VL >40 copies/mL) was previously assessed from all available penile lavage samples, and penile HSV-2 shedding status (DNA >50 copies/mL) was previously assessed among men seropositive for HSV-2.

Lavages were tested for pro-inflammatory cytokines (IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, and TNF-α) by a multiplex electrochemiluminescence immunoassay (Meso Scale Discovery Inc.). Samples were tested in duplicate within a plate, and were considered detectable if detected in both wells. Samples above detection were set to the ULQ per plate. Cytokines with <1% detectability were excluded from analyses: IFN-γ, IL-12p70, & IL-4.

Statistical Analyses

The probability of cytokine detection post-MC was examined relative to the pre-surgical visit, and analyses were stratified by plasma HIV viral suppression status (VL<400 copies/mL).

Analyses of penile HIV shedding were limited to men with detectable plasma HIV VL at enrollment. Prevalence risk ratios (PRRs) of HIV or HSV-2 shedding at post-surgical visits were estimated using Poisson regression models with generalized estimating equations and robust variance estimation. Multivariate models included adjustment for potential confounders: study visit and penile HIV/HSV-2 shedding status. Detectable $\log_{10}$ cytokine levels were compared by shedding status using rank sum tests.

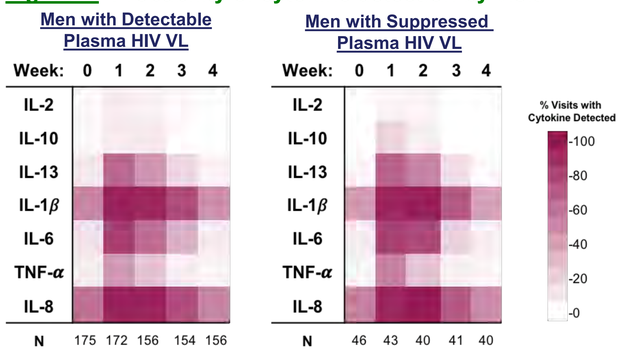
Results

Table 1. Baseline characteristics.

Characteristic	Detectable Plasma HIV VL (n=175)	Suppressed Plasma HIV VL (n=46)
Age, years		
<30	38.3% (67)	58.7% (27)
30-39	42.9% (75)	26.1% (12)
≥40	18.9% (33)	15.2% (7)
CD4⁺ T-cell count, cells/μL		
<200	41.1% (72)	39.1% (18)
200-299	46.3% (81)	47.8% (22)
≥300	12.6% (22)	13.0% (6)
GUD During Last 30 days		
No	84.6% (148)	93.5% (43)
Yes	15.4% (27)	6.5% (3)
Penile HSV-2 Shedding Status[#]		
No	91.3% (126)	86.1% (31)
Yes	8.7% (12)	13.9% (5)
Penile HIV Shedding Status		
No	90.1% (159)	97.8% (45)
Yes	9.1% (16)	2.2% (1)
Self-Report ART Use		
No	93.7% (164)	37.0% (17)
Yes	6.3% (11)	63.0% (29)
Median HIV $\log_{10}$ VL, c/mL (IQR)	4.6 (4.2-5.2)	-

[#] Penile HSV-2 shedding was not previously assessed in 37 HIV viremic and 10 HIV suppressed men.

Figure 1. Probability of cytokine detection by visit.



- Relative to baseline, detection of cytokines (except IL-2) was higher during weeks 1-3 post-MC.
- Baseline CD4⁺ T-cell count and genital ulcer disease (GUD) were not associated with cytokine detection.
- Certified healed wounds post-MC were associated with a decrease in cytokine detection (except IL-2 & IL-10).

Results (cont.)

Table 2. Cytokine detection associated with HIV and HSV-2 shedding from MC wounds.

Cytokine Detection		HIV Shedding Among Men with Detectable Plasma HIV VL: % HIV Shed (no. visits/total)	PRR (95% CI)	adj-PRR (95% CI)	HSV-2 Shedding Among All Men: % HSV-2 Shed (no. visits/total)	PRR (95% CI)	adj-PRR (95% CI)
IL-2	no	17.6% (106/601)	ref.	ref.	11.1% (66/595)	ref.	ref.
	yes	43.6% (14/32)	2.48 (1.51, 4.08) ‡	2.12 (1.57, 3.87) †	14.3% (4/28)	1.29 (0.43, 3.82)	1.19 (0.39, 3.61)
IL-10	no	17.4% (104/598)	ref.	ref.	11.0% (65/590)	ref.	ref.
	yes	45.7% (16/35)	2.63 (1.58, 4.38) ‡	1.87 (0.97, 3.58)	15.2% (5/33)	1.38 (0.53, 3.55)	1.20 (0.33, 1.71)
IL-13	no	13.4% (56/417)	ref.	ref.	10.6% (43/403)	ref.	ref.
	yes	29.6% (64/216)	2.21 (1.60, 3.03) ‡	1.82 (1.18, 2.80) ‡	12.3% (27/220)	1.15 (0.71, 1.87)	0.90 (0.52, 1.57)
IL-1β	no	4.3% (6/140)	ref.	ref.	6.2% (9/146)	ref.	ref.
	yes	23.1% (114/493)	5.40 (1.78, 16.37) ‡	3.73 (1.17, 11.94) ‡	12.8% (61/477)	2.07 (1.06, 4.07) †	1.66 (0.82, 3.39)
IL-6	no	10.5% (35/332)	ref.	ref.	8.8% (29/329)	ref.	ref.
	yes	28.2% (85/301)	2.68 (1.83, 3.92) ‡	2.23 (1.33, 3.72) ‡	14.0% (41/294)	1.58 (0.94, 2.67)	1.31 (0.71, 2.43)
TNF-α	no	13.9% (69/498)	ref.	ref.	11.2% (54/484)	ref.	ref.
	yes	37.8% (51/135)	2.73 (1.98, 3.75) ‡	2.17 (1.43, 3.28) ‡	11.5 % (16/139)	1.03 (0.55, 1.95)	0.87 (0.43, 1.76)
IL-8	no	4.6% (6/131)	ref.	ref.	4.4% (6/138)	ref.	ref.
	yes	22.7% (114/502)	4.96 (1.65, 14.89) ‡	3.48 (1.11, 10.94) †	13.2% (64/485)	3.04 (1.35, 6.80) ‡	2.56 (1.11, 5.88) †

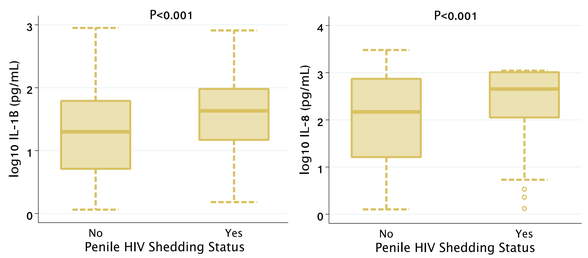
Multivariate models were adjusted for potential confounders determined a priori: study visit and penile HIV/HSV-2 shedding status. † P<0.05; ‡ P<0.01

Table 3. Association between the number of cytokines detected and HIV or HSV-2 shedding from MC wounds.

No. Cytokines Detected	HIV Shedding Among Men with Detectable Plasma HIV VL: % HIV Shed (no. visits/total)	PRR (95% CI)	adj-PRR (95% CI)	HSV-2 Shedding Among All Men: % HSV-2 Shed (no. visits/total)	PRR (95% CI)	adj-PRR (95% CI)
0-1	3.9% (6/153)	ref.	ref.	5.6% (9/161)	ref.	ref.
2-4	18.5% (66/356)	4.73 (1.54, 14.52) ‡	3.54 (1.11, 11.25) †	13.9% (47/338)	2.48 (1.25, 4.96) †	2.02 (0.99, 4.12)
5-7	37.7% (48/161)	9.61 (3.15, 29.35) ‡	7.34 (2.23, 24.09) ‡	10.9% (14/129)	1.94 (0.82, 4.58)	1.49 (0.58, 3.83)

Multivariate models were adjusted for potential confounders determined a priori: study visit and penile HIV/HSV-2 shedding status. † P<0.05; ‡ P<0.01

Figure 2. Cytokine levels by HIV shedding status.



- Among samples with detectable cytokines, higher levels of $\log_{10}$ IL-1β and IL-8 were associated with HIV shedding (Fig. 2), but no significant differences in cytokine concentrations were observed by HSV-2 shedding status.

Conclusions

Detectability of pro-inflammatory cytokines increased following MC but returned to baseline levels by 4 weeks.

Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-13 and TNF-α) were associated with penile HIV shedding, and IL-8 was also associated with penile HSV-2 shedding post-MC.

HIV- and HSV-2-infected men should be counseled on the risk of HIV and HSV-2 transmission prior to wound healing, highlighting the need for sexual abstinence during wound healing and consistent condom use thereafter.

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