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Genital Inflammation Predicts HIV-1 Shedding Independent of Plasma Viral Load and Systemic Inflammation

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Abstract

In women, genital HIV-1 RNA levels predict the risk of HIV-1 transmission independent of plasma viral load. To better understand the factors that contribute to genital HIV-1 shedding, we evaluated the relationships between genital and plasma cytokine concentrations and HIV-1 RNA levels. Vaginal, but not plasma, levels of interferon gamma-induced protein 10 (IP-10) were significantly associated with vaginal viral load, independent of plasma viral load. Thus, efforts to decrease HIV-1 transmission must take into account the role of local inflammation, which is not necessarily reflected in plasma measurements.

Keywords

HIV-1; inflammation; cytokine; genital; shedding; transmission

Introduction

The majority of HIV-1 infections worldwide are transmitted sexually, and the plasma viral load has commonly been used as a surrogate predictor of HIV-1 transmission risk¹. However, genital viral load has been shown to predict HIV-1 transmission risk independent of plasma viral levels², and genital shedding of HIV-1 can occur even in women with undetectable plasma levels of HIV-1³. Lower genital inflammation could influence viral

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shedding, and possibly provide an explanation for the sometimes imperfect correlation between plasma and genital viral loads.

Generalized immune activation is a hallmark of HIV-1 pathogenesis⁴. Whether this activation drives viral replication and shedding remains unknown, though expression of activation markers on lower genital T cells is associated with genital viral shedding and with mucosal CD4 depletion^{5,6}. Acute HIV-1 infection is associated with elevated levels of both pro-inflammatory (IL-6 and IL-12) and anti-inflammatory (IL-10) cytokines in the genital tract⁷, and elevated pro-inflammatory cytokines in cervicovaginal lavage (CVL) fluid have been associated with HIV-1 shedding^{8,9}. Thus, local inflammation could have a substantial influence on genital viral shedding by driving recruitment and/or activation of HIV-1-infected cells in the lower genital tract. We set out to attain a better understanding of how the systemic and genital inflammatory environments modulate HIV-1 transmission risk by examining the relationship between systemic and genital cytokine levels and viral loads.

Methods

Plasma and vaginal samples collected on the same day were evaluated from 48 HIV-1 seropositive women enrolled in a prospective cohort study in Mombasa, Kenya. None of the women had previously received or were receiving antiretroviral therapy at the time of sample collection. Vaginal samples were collected using a Dacron swab immersed in 1 mL of freezing medium (70% RPMI 1640, 20% fetal calf serum, 10% dimethyl sulfoxide). Full details of the participants and the sample collection procedures have been previously described^{10–12}. All participants provided informed consent, and the ethical review committees at the University of Washington, the Fred Hutchinson Cancer Research Center, and the University of Nairobi approved the study protocol.

Plasma and vaginal viral loads were determined by the Gen-Probe HIV-1 viral load assay¹⁰. The limit of detection for HIV-1 viral load was 100 copies/μL; values that were below the level of detection were assigned a value of 50 copies/mL. Cytokine concentrations were determined using the Milliplex MAP Human Cytokine/Chemokine Pre-mixed 26 Plex (Millipore Corporation, Billerica, MA) on Luminex 200 (Luminex Corporation, Austin, TX). The upper limit of cytokine detection was 10,000 pg/mL and the lower limit was 1.52 pg/mL. Samples that were less than the lower limit of detection were assigned a value of 0.76 pg/mL. No analyses were performed on cytokines in which >95% of the observations were below the level of detection (IL-2, IL-3, IL-4, IL-5, and TNF-β in plasma). For sexually transmitted infection (STI) detection, a vaginal saline wet mount was examined for the presence of yeast and motile *Trichomonas vaginalis* parasites, and vaginal Gram stained slides were evaluated for the presence of bacterial vaginosis using Nugent's criteria¹³. Culture for *Neisseria gonorrhoeae* was performed on modified Thayer-Martin media. Genital ulcer disease was diagnosed based on clinician examination.

Statistical analyses were performed in SPSS version 20 (IBM Corp.). Cytokine levels were log₁₀ transformed; if more than 20% of the measurements were below the lower limit of detection, a dichotomous variable was used reflecting levels at or below versus above the lower limit of detection. Linear regression was used to assess the relationships between the log₁₀plasma HIV-1 RNA and plasma cytokine levels, log₁₀vaginal HIV-1 RNA and vaginal cytokine levels, log₁₀plasma HIV-1 RNA and vaginal cytokine levels, and log₁₀vaginal HIV-1 RNA and plasma cytokine levels. Regression diagnostics including p-plots and residual plots were performed to assess the fit of the models; the linear regression models fit well. Adjustment for multiple comparisons was performed by controlling for a false discovery rate of 5%. The relationship between plasma and vaginal cytokine levels was assessed using Spearman's rank correlation coefficients.

Results

The median plasma viral load was 4.69 log₁₀copies/mL (interquartile range (IQR) 4.14,5.12), and the median genital viral load was 2.47 log₁₀copies/mL with 21% below the limit of detection (IQR 1.97, 3.77). Plasma viral load was strongly correlated with vaginal viral load (Pearson's correlation coefficient = 0.47, p=0.001).

Vaginal cytokine concentrations were highest for IL-8, and more than 20% of observations were below the limit of detection for many cytokines (Table 1). Vaginal levels of several pro-inflammatory cytokines and chemokines (IP-10, MIP-1β, IL-7, IFN-α2, G-CSF, IL-6, IL-8, and TNF-α) were significantly associated with the vaginal viral load. Only levels of IP-10, a chemokine secreted by monocytes, endothelial cells, and fibroblasts in response to IFN-γ, remained significantly associated with shedding after controlling for a false discovery rate of 5% (Table 1). For every 1 log₁₀ pg/mL increase in vaginal there was a 0.744 log₁₀ increase in vaginal HIV-1 RNA.

Since plasma HIV-1 RNA levels were significantly associated with vaginal HIV-1 RNA levels, we evaluated whether cytokine levels predicted vaginal HIV-1 RNA levels after adjusting for plasma HIV-1 RNA levels. Vaginal levels of IP-10, MIP-1β, IL-7, IFN-α2, IL-8, and TNF-α remained significantly associated with vaginal HIV-1 RNA levels after adjustment for plasma HIV-1 RNA levels, though only IP-10 remaining significant after controlling for a false discovery rate of 5% (Table 1). Finally, since hormonal contraceptive (HC) use and STI have been associated with viral shedding^{14,15} and may be associated with genital inflammation, we adjusted for plasma log₁₀ HIV-1 RNA, HC use, and STIs. STI screening was limited to bacterial vaginosis, *Trichomonas vaginalis*, yeast, GUD by clinician exam, and *N. gonorrhoeae* by culture. We found that vaginal levels of IP-10, MIP-1β, IL-7, IL-8, and TNF-α were significantly associated with vaginal HIV-1 RNA levels prior to controlling for a false discovery rate of 5%, though none of these associations were significant after adjusting for multiple comparisons (Table 1). There were no statistically significant associations between vaginal cytokine concentrations and plasma HIV-1 RNA levels (p > 0.08 for all cytokines).

With the exception of IP-10, plasma cytokines levels were lower than vaginal cytokine levels, and >20% of the values were below the limit of detection for the several cytokines (Table 2). The only plasma cytokine that was significantly associated with plasma HIV-1 RNA levels was IP-10 (regression coefficient 0.728, 95% CI 0.047, 1.409, p = 0.04, Table 2), and this association was not significant when controlling for a false discovery rate of 5%. There were no statistically significant relationships between any of the plasma cytokines and vaginal HIV-1 RNA (p > 0.08 for all cytokines). In fact, plasma and vaginal cytokine levels were generally not statistically significantly associated. Only IL-10 (Spearman's rho = 0.3449, p = 0.016) and IL-15 (Spearman's rho = 0.386, p = 0.007) levels were significantly associated between the plasma and vaginal compartments, but these associations were driven entirely by the fact that only 3 subjects had levels of IL-10 and one individual had levels of IL-15 above the limit of detection in the plasma.

Discussion

We report here that local inflammation is associated with increased genital HIV-1 RNA shedding, independent of plasma viral load. Thus, these findings indicate limitations of using plasma viral load to assess transmission risk, and highlight the importance of considering the impact of the local inflammatory environment in modulating transmission. Local inflammation was associated with substantial alterations in the genital viral load, which could result in increased risk of transmission. For instance, in a recent study of

serodiscordant couples in Africa, each 1 log₁₀ increase in endocervical viral load was associated with a 1.67-fold relative risk of HIV-1 transmission to the uninfected partner (95% CI 1.60, 3.04)².

Chronic inflammation is a hallmark of HIV-1 disease progression⁴, yet few prior studies have simultaneously evaluated the systemic and genital inflammatory environment in conjunction with HIV-1 viral load. Jaspan et al. observed that the frequency of activated T cells in the blood correlated with that in lower genital tract of HIV-1 infected women, and that the latter predicted HIV-1 shedding⁶. While we similarly found that concentrations of several pro-inflammatory cytokines predicted vaginal shedding (though only IP-10 remained significant after adjusting for multiple comparisons), we found that the systemic concentrations of pro-inflammatory cytokines were not predictive of genital concentrations, nor were they associated with genital HIV-1 shedding. Together, these data indicate that measurements of systemic inflammation cannot necessarily be used to predict the genital tract inflammatory environment or shedding.

If the systemic inflammatory environment does not fully predict the genital environment, then it is important to understand what factors modulate genital inflammation and viral shedding. STIs are associated with genital inflammation and with increased HIV-1 shedding and transmission risk¹⁵. Similarly, use of HCs are associated with genital inflammation^{16,17}, and several reports have now demonstrated that HCs increase HIV-1 transmission risk, though conflicting data exist^{18,19}. After adjusting for the plasma viral load, use of HCs, and the presence of several STIs, increased concentrations of several vaginal pro-inflammatory cytokines were associated with HIV-1 shedding, though none of these associations remained statistically significant after adjusting for multiple comparisons. Since inflammation can persist long after STI symptom resolution²⁰, post-STI inflammation and/or STIs that we did not adequately measure (such as subclinical HSV infection or reactivation) could explain the weak associations between inflammation and shedding in this model. Future studies will be necessary to more definitively establish whether even subclinical levels of genital inflammation increase transmission risk.

Significant strengths of this study include the use of matched plasma and vaginal collections from a well-characterized study of HIV-1 infection in women. Limitations of this analysis include the relatively small sample size, and the fact that some STIs that could have influenced genital inflammation were not assessed. In addition, variation in the volume of vaginal secretions on each swab could have accentuated the observed associations between concentrations of vaginal inflammatory markers and HIV-1 RNA. Detection of cytokines in vaginal swabs, which are reliable for detection of HIV-1 RNA¹², yielded lower cytokine concentrations than observed in some prior studies of CVL^{8,9}, but were in agreement with other studies^{7,21}. Given the large number of analytes, the associations between inflammatory cytokines and shedding were significantly attenuated after controlling for a false discovery rate of 5%. However, this may be an overly conservative approach to analyzing these data, as many cytokines are positively correlated. Additional studies, with larger numbers of subjects from different risk groups and geographic regions, will be needed to confirm the observed associations between local inflammation and shedding.

These data bolster and extend previous reports that local inflammation is associated with HIV-1 shedding^{5,6,8,9,21}. Mitchell et al. found that CVL IL-1 β , a potent pro-inflammatory cytokine produced by macrophages, and IL-8, a chemokine produced by macrophages, epithelial cells, and other cells, were significantly associated with CVL HIV-1 RNA independent of plasma viral load. The relationship with IL-1 β , but not IL-8, was attenuated after adjusting for STI, suggesting that HIV itself and/or non-infectious cases of inflammation may play a role in cervicovaginal HIV-1 shedding²¹. Similarly, Spear et al.

and Makura et al. found that several pro-inflammatory cytokines, including IL-6, were significantly associated with shedding^{8,9}. Genital pro-inflammatory cells were also associated with shedding in two studies^{5,6}, though in one, IL-6 and IL-8, which are secreted primarily by macrophages and epithelial cells, did not predict shedding⁶. The differences between studies in precisely which cytokines or cells predict shedding could reflect the fact that the inflammatory mechanisms leading to T cell activation vs. IL-6/IL-8 production could be distinct and independently influence shedding. Furthermore, differences in cohorts and in sampling methods could account for some of these differences.

Together, these data highlight the fact that the genital environment may have a substantial influence of HIV-1 transmission. Genital inflammation is associated with CD4+ T cell depletion both systemically and in the lower genital tract^{6,7}. Here we show that genital inflammation is also associated with HIV-1 shedding in the genital tract, independent of systemic inflammation and viral load. Thus, efforts to decrease HIV-1 transmission must take into account the role of local inflammation and the fact that the local environment is not necessarily reflected in plasma measurements.

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Table 1
Associations between vaginal HIV-1 RNA and vaginal cytokine levels, sorted by unadjusted p-value

Cytokine	Median conc. (pg/mL)	IQR	% above LOD	Unadjusted			Adjusted for plasma log ₁₀ HIV-1 RNA			Adjusted for plasma log ₁₀ HIV-1 RNA, HC use, and presence of STI ⁺		
				Reg. Coef	95% CI	p- value [#]	Reg. Coef.	95% CI	p- value [#]	Reg. Coef	95% CI	p- value [#]
IP-10 [*]	199	58.8, 644.6	100	0.744	(0.352, 1.136)	<0.001	0.692	(0.346, 1.037)	<0.001	0.613	(0.224, 1.003)	0.003
MIP-1β	7.68	3.03, 22.3	90	0.781	(0.231, 1.332)	0.006	0.643	(0.138, 1.149)	0.014	0.579	(0.038, 1.121)	0.04
IL-7 [^]	0.76	0.76, 2.62	44	0.919	(0.254, 1.583)	0.008	0.826	(0.231, 1.420)	0.008	0.687	(0.004, 1.369)	0.05
IFN-α2 [*]	3.48	1.61, 6.45	81	0.826	(0.127, 1.525)	0.022	0.712	(0.081, 1.342)	0.028	0.626	(-0.067, 1.320)	0.08
G-CSF [*]	39.6	5.05, 291	81	0.325	(0.025, 0.625)	0.035	0.248	(-0.028, 0.524)	0.08	0.208	(-0.091, 0.507)	0.2
IL-6 [^]	0.76	0.76, 11.34	48	0.73	(0.049, 1.412)	0.036	0.465	(-0.183, 1.114)	0.2	0.463	(-0.215, 1.142)	0.2
IL-8 [*]	893	269, 3287	98	0.428	(0.018, 0.838)	0.041	0.450	(0.091, 0.809)	0.015	0.423	(0.042, 0.804)	0.03
TNF-α [^]	2.06	0.76, 7.11	63	0.733	(0.022, 1.444)	0.044	0.911	(0.299, 1.523)	0.004	0.845	(0.213, 1.477)	0.01
Eotaxin [*]	30.2	13.0, 50.2	98	0.759	(-0.004, 1.522)	0.051						
IL-15 [^]	0.76	0.76, 0.76	15	0.952	(-0.011, 1.915)	0.053						
IL-4 [^]	2.86	0.76, 5.99	73	0.751	(-0.038, 1.539)	0.062						
GM-CSF [^]	0.76	0.76, 1.87	31	0.648	(-0.095, 1.390)	0.09						
IL-1α [*]	349	94.8, 713	100	0.472	(-0.131, 1.075)	0.1						
IL-1β [^]	7.05	0.76, 21.3	69	0.571	(-0.193, 1.334)	0.1						
IL-5 [^]	0.76	0.76, 0.76	6	1.075	(-0.352, 2.502)	0.1						
IL-12p70 [^]	0.76	0.76, 1.83	27	0.593	(-0.187, 1.372)	0.1						
IFN-γ [^]	1.15	0.76, 4.45	50	0.515	(-0.184, 1.213)	0.2						
MIP-1α [^]	2.76	0.76, 8.11	63	0.495	(-0.235, 1.224)	0.2						
IL-10 [^]	0.76	0.76, 0.76	17	0.489	(-0.452, 1.429)	0.3						
IL-17 [^]	1.58	0.76, 4.15	50	0.327	(-0.403, 1.057)	0.4						
MCP-1 [^]	4.11	0.76, 38.4	71	0.355	(-0.438, 1.147)	0.4						
IL-3 [^]	0.76	0.76, 1.87	29	0.241	(-0.555, 1.038)	0.5						

Cytokine	Median conc. (pg/mL)	IQR	% above LOD	Unadjusted			Adjusted for plasma log ₁₀ HIV-1 RNA			Adjusted for plasma log ₁₀ HIV-1 RNA, HC use, and presence of STI ⁺		
				Reg. Coef	95% CI	p-value [#]	Reg. Coef.	95% CI	p-value [#]	Reg. Coef	95% CI	p-value [#]
IL-12p40 [^]	3.39	0.76, 8.11	60	0.225	(-0.518, 0.968)	0.5						
TNF-β [^]	0.76	0.76, 0.76	8	0.467	(-0.807, 1.741)	0.5						
IL-2 [^]	0.76	0.76, 0.76	10	0.311	(-0.845, 1.468)	0.6						
IL-13 [^]	0.76	0.76, 0.76	13	-0.083	(-1.155, 0.988)	0.9						

⁺ Abbreviations: LOD: Limit above detection; HC: hormonal contraceptives; STI: sexually transmitted infection including genital ulcer disease, gonorrhea, candida, bacterial vaginosis, chlamydia, or trichomoniasis; Reg Coef., regression coefficient; CI: Confidence interval

^{*} log10 transformed values.

[^] Dichotomized variable, testing for > lower limit

[#] Nominal p-value is reported, with significant associations indicated by bold type based on p-value < 0.05. Those that remained significant after controlling for a false discovery rate of 5% are underlined, with the assumption of 26 tests for each set of calculations (both univariate and multivariate).

Table 2

Associations between Plasma HIV-1 RNA and plasma cytokine levels

Cytokine	Median (pg/mL)	IQR	% above LOD	Reg. Coef. ⁺	95% CI	p-value [#]
IP-10 [*]	1459	836, 3265	100	0.73	(0.047, 1.409)	0.04
IL-1α [^]	4.475	0.76, 14.1	58	0.52	(-0.032, 1.070)	0.06
IL-17 [^]	0.76	0.76, 0.76	10	0.80	(-0.099, 1.688)	0.08
MCP-1 [*]	87.7	62.2, 136	100	0.59	(-0.075, 1.255)	0.08
Eotaxin [*]	21.2	15.9, 32.7	100	0.80	(-0.158, 1.755)	0.1
GM-CSF [*]	12.7	5.98, 49.0	94	0.34	(-0.064, 0.754)	0.1
IL-6 [^]	0.76	0.76, 0.76	17	-0.61	(-1.345, 0.126)	0.1
IL-12p70 [^]	0.76	0.76, 1.18	25	0.49	(-0.150, 1.121)	0.1
TNF-α [*]	2.13	1.73, 3.15	90	0.50	(-0.176, 1.176)	0.1
IFN-α2 [^]	0.76	0.76, 2.31	25	0.45	(-0.193, 1.083)	0.2
IFN-γ [^]	0.76	0.76, 1.75	27	0.40	(-0.224, 1.024)	0.2
MIP-1α [*]	25.8	12.0, 50.3	92	0.20	(-0.112, 0.511)	0.2
IL-1β [^]	0.76	0.76, 0.76	10	0.450	(-0.461, 1.367)	0.3
MIP-1β [*]	31.0	14.2, 51.4	100	0.34	(-0.285, 0.966)	0.3
IL-7 [^]	0.76	0.76, 0.76	6	0.50	(-0.657, 1.656)	0.4
IL-12p40 [^]	0.76	0.76, 1.57	25	0.26	(-0.388, 0.907)	0.4
G-CSF [*]	8.84	5.95, 12.7	96	0.27	(-0.536, 1.075)	0.5
IL-10 [^]	0.76	0.76, 0.76	6	0.34	(-0.826, 1.497)	0.6
IL-8 [*]	18.2	5.05, 43.3	94	0.06	(-0.322, 0.446)	0.7

⁺ Abbreviations: LOD: Limit of detection; Reg Coef: regression coefficient, CI: Confidence interval^{*} log10 transformed values.[^] Dichotomized variable, testing for > lower limit[#] Nominal p-value is reported, with significant associations indicated by bold type based on p-value < 0.05. Those that remained significant after controlling for a false discovery rate of 5% are underlined, with the assumption of 26 tests.