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Genital levels of soluble immune factors with anti-HIV activity may correlate with increased HIV susceptibility

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Despite the scale of the HIV pandemic, mucosal innate immune defenses prevent HIV infection after most sexual exposures. Several soluble immune factors with *in vitro* anti-HIV activity are present at the genital mucosa in physiologically relevant concentrations, including SLPI, defensins and RANTES[1]. However, the impact of such factors on *in vivo* HIV susceptibility has been poorly defined[2]. A better understanding of the mucosal immune correlates of HIV susceptibility is needed, particularly since several potential microbicide candidates with *in vitro* anti-HIV activity have been unexpectedly associated with an increased HIV incidence in phase 3 trials[3]. Genital RANTES levels are increased in HIV-exposed, persistently seronegative (HEPS) women[4-6], suggesting a possible intermediate endpoint for reduced HIV susceptibility. However, RANTES is produced by activated immune cells, including HIV-susceptible CD4+ T cells, and levels are increased in conditions such as bacterial vaginosis (BV) that may increase HIV susceptibility[6]. Therefore, we examined the correlation between genital levels of soluble immune factors with anti-HIV activity, including RANTES, and cervical immune cell populations in high risk, HIV-uninfected women.

Participants were enrolled through a female sex worker (FSW) clinic in Nairobi, Kenya[7]. Informed consent was obtained, and the protocol was approved by Research Ethics Boards at the University of Toronto, the University of Manitoba and Kenyatta National Hospital (Nairobi, Kenya). A cervico-vaginal lavage (CVL), cervical cytobrush specimen and scraping of the external cervical os (Benzi Jinshuo Applicator Co., Liaoning, China) were collected, and

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CONTRIBUTIONS

Experimental design and planning: RK, AR, TH, CW, TBB, FAP, JK, WJ. Obtained funding: RK, FAP.
Recruitment, sampling and immune assays: AR, CW.
Data analysis: RK, AR.
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diagnostic testing performed for HIV, *T. vaginalis*, *N. gonorrhoeae*, *C. trachomatis*, syphilis, herpes simplex type 2 (HSV-2) infection/shedding, cytomegalovirus (CMV) infection/shedding and bacterial vaginosis (BV)[7]. All infections were treated according to Kenyan national guidelines. Cervical samples were filtered, washed and stained with a panel of dendritic cell (DC) and T-cell markers, including CD69-FITC, CCR5-PE, CD3-PerCP, CD4-APC and CD1a-FITC, (BD Pharmingen, San Jose, CA, USA); TLR9-PE (Imgenex, San Diego, CA, USA); DC-SIGN-APC (eBioscience, San Diego, CA, USA); and isotype controls. Populations were enumerated by flow cytometry. Levels of RANTES and other cytokines/chemokines were assayed by Cytokine Bead Array (CBA, BD Biosciences, San Diego, CA, USA). SLPI levels were measured by ELISA (Quantikine Human SLPI kit, R&D Systems, Minneapolis, MN).

Fifty-five HIV-uninfected FSWs participated in the study. RANTES was detectable in the CVL of 51/55 participants (93%). The median RANTES level was 12.9 pg/ml (range; 0-743.3pg/ml). There was s strong positive correlation between RANTES levels and the number of cervical CD4 T cells (r=0.53; P=0.00006; Figure 1a), cervical CD4+ T cells expressing the HIV co-receptor CCR5 (r=0.31; P=0.028), cervical CD8+ T cells (r=0.52; P=0.00005), immature dendritic cells (iDCs; r=0.38; P=0.006; Figure 1b), and iDCs expressing the TLR9 receptor (r=0.40; P=0.004). Genital RANTES levels also correlated with increased pro-inflammatory cytokines (IL1, IL6, IL8 and TNF alpha; all $P \leq 0.01$) and SLPI (r=0.34; P=0.018).

Classical STIs were uncommon (*N. gonorrheae*, n=1; *C. trachomatis*, n=0; syphilis, n=2; *T. vaginalis*, n=4) and were not associated with differences in RANTES levels (data not shown). Although most participants were HSV-2 infected (42/55; 76%), RANTES levels did not vary with HSV-2 infection status (1.14 log10 pg/ml, infected; vs 1.28, uninfected; P=0.3). HSV-2 shedding was not detected in any participants. BV was present in 15/33 participants with an available Gram stain (45%). Genital RANTES levels tended to be higher in BV (1.24 vs 0.95 log10 pg/ml; P=0.054), and were positively correlated with the Nugent score (r=0.42; P=0.016).

Low rates of HIV acquisition after sexual exposure complicate the performance of HIV microbicide trials[8], and compounds with *in vitro* anti-HIV activity may actually increase HIV susceptibility[3]. Well validated mucosal immune correlates of HIV susceptibility would permit the establishment of much-needed intermediate endpoints for microbicide safety and efficacy studies[9], but these correlates remain poorly defined. We demonstrate that increased genital levels of RANTES, a soluble immune factor with putative anti-HIV activity, could actually indicate an increase in HIV susceptibility as reflected by the number of HIV-susceptible target cells present in the cervical mucosa. Therefore, considerable caution must be exercised when using genital levels of this or other soluble immune factors to make assumptions regarding HIV susceptibility. Previous studies demonstrating increased RANTES levels in the genital tract of HEPS women were not able to control for the increased prevalence of BV and/or other genital co-infections that may result from the high risk sexual practices themselves, rather than representing a mechanism of immune protection from HIV acquisition during unprotected sex. Future studies should control for the effects of a wide array of genital infections, including classical bacterial STIs and HSV2, and for disturbances in the vaginal flora.

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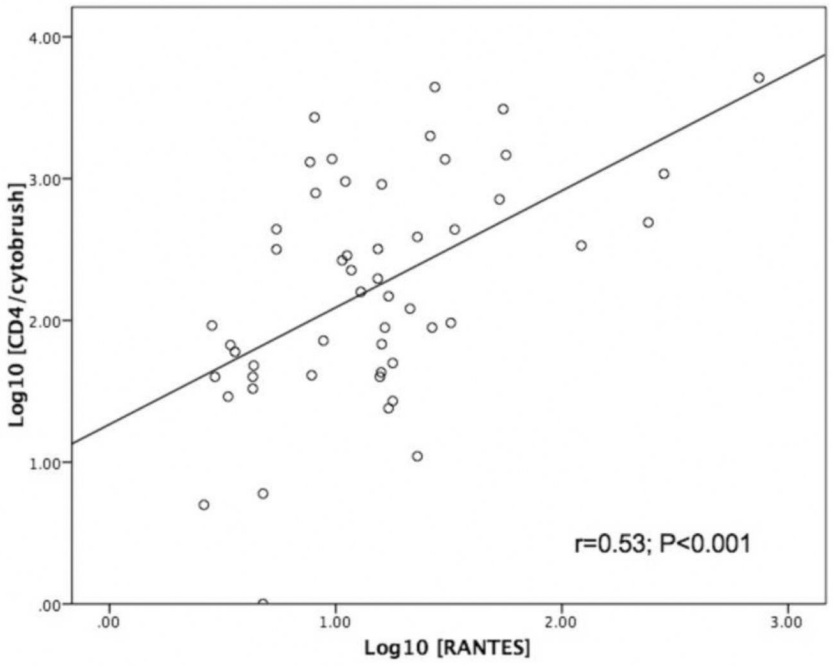
a)



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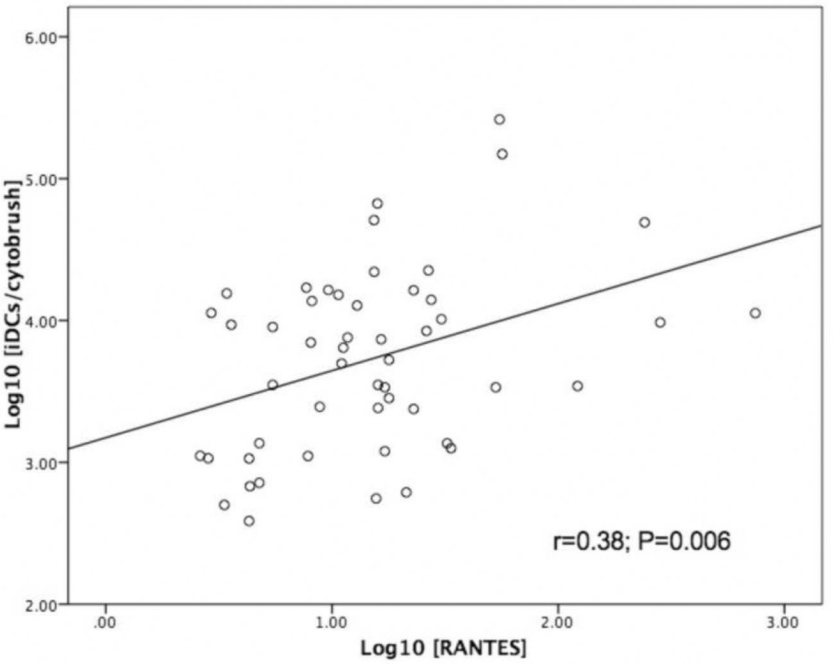

Figure 1. Correlation of genital RANTES levels with increased numbers of HIV-susceptible cells in the cervical mucosa

Log10 transformed levels of RANTES in cervico-vaginal lavage samples from high-risk Kenyan women were positively correlated with numbers of cervical CD4+ T cells (Figure 1a) and of cervical immature dendritic cells (Figure 1b).