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Systemic and Mucosal Differences in HIV Burden, Immune and Therapeutic Responses

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Abstract

Background—Mucosal tissues represent major targets for HIV transmission, but differ in susceptibility and reservoir function by unknown mechanisms.

Methods—In a cross-sectional study, HIV RNA and infectious virus were compared between oral and genital compartments and blood in HIV-infected women, in association with clinical parameters, co-pathogens and putative innate and adaptive HIV inhibitors.

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The Woman's Interagency HIV Study Protocol was reviewed and approved by the institutional review boards at each participating center, and written and informed consent was obtained from all patients. Human experimentation guidelines of the U.S. Department of Health and Human Services were followed in the conduct of this research. The authors report no potential conflicts of interest.

Results—HIV RNA was detectable in 24.5% of women from all 3 compartments, whereas 45% had RNA in only one or two sites. By comparison, infectious HIV, present in blood of the majority, was rare in mucosal sites. Innate mediators, SLPI and TSP, were highest in mucosae. Highly active antiretroviral therapy (HAART) was associated with an 80% decreased probability of shedding. Multivariate logistic regression models revealed that mucosal HIV RNA was associated with higher plasma RNA, infectious virus, and total mucosal IgA, but not IgG. There was a 37-fold increased probability of detecting RNA in both genital and oral specimens ($P=0.008$; $P=0.02$, respectively) among women in highest vs lowest IgA tertiles.

Conclusions—Mucosal sites exhibit distinct characteristics of infectious HIV, viral shedding and responses to therapy, dependent upon both systemic and local factors. Of the putative innate and adaptive mucosal defense factors examined, only IgA was associated with HIV RNA shedding. However, rather than being protective, there was a striking increase in probability of detectable HIV RNA shedding in women with highest total IgA.

Keywords

HIV-1; mucosa; innate immunity; adaptive immunity; IgA; SLPI

Introduction

The primary site of transmission and acquisition of human immunodeficiency virus-1 (HIV) in both women and men is through the genital mucosa¹. Enhanced levels of HIV in genital mucosal secretions have been associated with multiple factors including ulcers, local infections, inflammation and plasma HIV RNA^{2, 3}. By comparison, epidemiologic studies indicate that oral mucosa is an uncommon conduit for either transmission or acquisition of HIV⁴⁻⁶, unlike herpesviruses (HSV1,2) and cytomegalovirus (CMV) that are transmitted by both oral and genital routes, and Epstein-Barr virus (EBV) and human herpesvirus-6 (HHV6) transmitted orally⁷. However, bleeding in the oral cavity was suspected in HIV transmission in pre-masticated food from caregiver-to-infant⁸, confounding the evidence. The relative infrequency of oral transmission may reflect a paucity of HIV replication locally, unique mucosal architecture, and/or presence of antiviral molecules^{4, 9-12}, but differences in parameters underlying rates of genital and oral mucosal transmission are not well understood.

Multiple local and systemic virologic and immunologic differences must be considered in deciphering susceptibility and resistance profiles of mucosal sites relative to HIV transmission, including viral load, CD4⁺ T cell counts, co-infections, antiviral therapy, innate and adaptive immune responses. Among the endogenous innate mucosal factors that may differentially impact on HIV transmission and acquisition are mucins, defensins, secretory leukocyte protease inhibitor (SLPI) and thrombospondin (TSP-1), which have been shown to exhibit anti-HIV activity¹³, as well as adaptive immune response mediators and HIV-specific antibodies. Differences in these molecules among mucosal compartments or in peripheral blood and their relationship to HIV remain understudied, as does the relative impact of antiviral therapy. As the prevalence of HIV infection in women continues to increase, it remains critical to understand gender-specific susceptibilities, including routes of infection, localization of viral replication, role of innate factors, co-infections, potential compartmentalization of antiretroviral therapies (ART) and vaccine targeting. This cross-sectional study of HIV-infected women was designed to: (1) compare HIV RNA (viral shedding) and infectious virus in two mucosal compartments, oral cavity and genital tract, with these quantifiable measures of viral burden in peripheral blood, and (2) to identify selected influential intrinsic and extrinsic factors that may differ between compartments to influence HIV levels.

Methods

Subjects

This was a cross-sectional substudy within DATRI 0093. After recruitment of the first 225 women from Women's Interagency HIV Study(WIHS), a multicenter, prospective study of HIV infection¹⁴, the study was amended to enroll an additional 115 women(DATRI 009b) to include oral mucosa and expanded immunologic panel. Nonpregnant 19-45yr-old infected women with intact uterus and cervix were invited to participate under separate informed consent, and excluded if presenting with AIDS-defining illness, had begun or changed ART within one month or reported coitus, douching, spermicide or antimicrobial therapy in prior 48h. Women were evaluated at a single visit (6-month WIHS visit) that included collection of data regarding demographics, medications, illicit drug use, limited physical, oral and vaginal examinations, and collection of blood, oral and vaginal specimens.

Peripheral blood

Blood was collected in sodium citrate cell preparation tubes(Vacutainer, Becton-Dickinson) and mononuclear cells and plasma separated by centrifugation(400×g, 10min). Plasma was centrifuged(1000×g, 10min) before storage(-70°C). Analyses of lymphocytes for CD4⁺ and CD8⁺ were performed using standardized protocols³.

Oral cavity

Specimens were sequentially obtained: (1) 4 swabs (buccal mucosa, tongue, floor of mouth, palate) and swabs of any active lesions, (2) buccal cytobrush for HIV culture, (3) unstimulated saliva collected in tubes on ice over 10min, and (4) expectorated gargle obtained with 7.2ml sterile distilled H₂O. A dental examiner assessed mucosal and periodontal health in a subset. Swabs were cultured for *Candida albicans*. For culture of HIV, HSV, CMV and respiratory viruses(influenza, parainfluenza, respiratory syncytial virus, adenovirus), specimens were placed in vials containing 2ml RPMI1640 with L-glutamine, 20%FBS, 100U/ml penicillin, 100μl streptomycin and 160U/ml nystatin. For HIV PCR, swabs in medium with 4M guanidine isothiocyanate and mercaptoethanol were frozen until tested³.

Saliva was unspun or centrifuged (1500×g, 20min, 4°C). Unspun saliva was used for HIV RNA and culture, Human Papillomavirus(HPV) PCR and blood(Chemstrip-OB, Boehringer-Mannheim Diagnostics). Supernatants were diluted in Ca⁺⁺Mg⁺⁺-free PBS(1:3) and frozen until determination of immunoglobulins(Ig), SLPI and TSP-1. Gargle mixed with 10× RPMI1640 and 20%FBS with antibiotics was aliquoted for EBV, HHV6 and HHV8 assays^{15, 16}.

Genital tract

Following cervicovaginal lavage(CVL) with 10ml sterile PBS, 4 endocervical swabs[for *Candida albicans*, pH, potassium hydroxide(KOH), amine odor test, microscopy, HIV RNA] and one cytobrush(HIV culture) were collected³. Mixed swabs were tested for HSV PCR¹⁷ and cultured for HSV1, HSV2, CMV, adenovirus, enterovirus and *Varicella zoster*³. If present, genital lesions were swabbed for pathogen identification. Unspun CVL was tested for blood and semen(SemaTest, Humagen Fertility Diagnostics, Charlottesville, VA) and frozen for HIV RNA and HPV PCR¹⁸ or centrifuged(400×g, 10min) for Ig, SLPI and TSP-1. *Trichomonas vaginalis* was assessed by wet mount and bacterial vaginosis by Amsel¹⁹.

HIV-1 evaluations

Semi-quantitative HIV cultures of blood and qualitative HIV cultures of genital(unspun CVL, swab) and oral(unspun saliva, swab) specimens were performed within 6hr by protocol²⁰. Infectious virus was expressed as infectious units/10⁶ cells(IUPM). For qualitative analysis, blood IUPM values >0.3219 were considered positive and ≤0.3219 negative³.

HIV RNA was assayed in oral swab, plasma, CVL and unspun saliva with Nuclisens(Organon Teknika Corporation, Raleigh, NC), while cervical swabs were assayed with Roche Amplicor HIV-1 Monitor test(Roche Molecular Systems, Branchburg, NJ). Lower limit of detection(LLD) for plasma and cervical swabs was 400 copies/ml, and CVL, oral swabs and saliva had LLD of 80 copies/ml²¹.

Immunologic evaluations

Centrifuged saliva(1:3 in PBS), CVL and plasma were tested for SLPI and TSP-1 by ELISA(R&D Systems, Minneapolis, MN) or as described¹². Total IgG and IgA and HIV-specific antibodies were determined by capture ELISA²². IgG and IgA anti-gp120 were measured against standard curves of polyclonal secretory IgA or serum(Moni-Trol ES, Baxter, Stone Mountain, GA) with high anti-HIV-1 Ig as positive control and mucosal secretions and sera of uninfected individuals as negative controls. The cutoff(nonspecific) was set at 100ng/ml.

Study definitions

For our analyses, HIV shedding was defined as presence of HIV RNA in oral swab or saliva and in genital swab or CVL using LLD above. We used DHHS/Kaiser Panel[DHHS/Kaiser 2004] guidelines to define HAART usage: (a) ≥2 NRTIs in combination with ≥1 PI or one NNRTI(88% of observations classified as HAART); (b) one NRTI in combination with ≥1 PI and ≥1 NNRTI(5%); (c) regimen containing ritonavir and saquinavir in combination with one NRTI and no NNRTIs(1%); and (d) abacavir or tenofovir containing regimen of ≥3 NRTIs in absence of PI and NNRTI(6%), except for three-NRTI regimens consisting of: abacavir+tenofovir+lamivudine or didanosine+tenofovir+lamivudine.

Statistical analysis

Frequency distributions were calculated for categorical variables and median(range) for continuous variables. Associations among detection of HIV RNA in three compartments(oral, genital, blood) were assessed using a log-linear model; models were analyzed in total sample and also stratified by ART. HIV RNA was considered detectable in mucosal compartments if either swab or fluid was above LLD; women with both tests not completed were treated as unknown. To compare median levels of innate and adaptive molecules between compartments, Wilcoxon signed rank tests were used. Using logistic regression to assess relationships between HIV RNA in oral and genital compartments with clinical and laboratory assessments, odds ratios(ORs) and 95% confidence limits were calculated. The relationship between each variable and presence of HIV RNA in oral cavity and genital tract was analyzed in multivariate logistic regression models adjusting for ART(none, monotherapy, combination therapy, HAART) and plasma HIV RNA(<400, 400-10,000, ≥10,000 copies/ml) or ART only where appropriate. Additional multivariate models adjusted for CD4⁺ T cells(<200, 200-<500, 500⁺ cells/mm³). In logistic regression models, SLPI, TSP, IgA and IgG were categorized by tertile. Relationships between total IgA and HIV RNA within each compartment were estimated by Spearman rank correlation coefficients. Two-sided hypotheses were used throughout, testing at 5% significance level.

Results

HIV RNA and infectious HIV in blood, oral cavity and genital tract

Demographic and clinical characteristics of the WIHS cohort, including this subset (n=115) 3, 14 are summarized in Supplemental Table 1. The majority were African American (57%) and Hispanic (20%) women who reported heterosexual contact as primary risk factor for transmission; only 24% reported injection drug use (IDU). Most had detectable plasma viral burden with approximately one third having HIV RNA levels <400, one third from 400-10,000 and the remainder >10,000 copies/ml (Supplemental Table 1).

We evaluated presence of HIV RNA across compartments among the 106 women with available data in oral, blood and genital tract compartments in relationship to antiretroviral therapy (Table 1). Overall, HIV RNA was detectable in all three compartments in 24.5% of women and in none of the compartments for 29.3% (Table 1, Total). Women not receiving ART were most likely to have detectable HIV RNA in all three (35.1%) or any compartment (86.5%). Among women with undetectable plasma RNA, 8% had detectable RNA in genital secretions and 3% in oral cavity (data not shown). Typically, HIV RNA levels were lower in genital and oral compartments than plasma [genital RNA/plasma RNA, median(range) =15.9%(0.3-937.4%); oral RNA/plasma RNA =8.6%(0.1-300%)]. While HIV RNA was detectable in the oral compartment of 30%, none had infectious virus and in genital secretions, 40% had detectable HIV RNA, but only two had detectable infectious virus (Supplemental Table 1), irrespective of therapy, demonstrating differential dynamics of infectious HIV in systemic and mucosal compartments.

Mucosal and systemic innate and adaptive immune factors and HIV

To explore additional factors that may contribute to disparate HIV detection in mucosal sites compared to blood, we monitored two innate factors reported to inhibit HIV, SLPI and TSP-1 10 13, 23. Although TSP-1 was highest in blood (Fig. 1A; median 193ng/ml [range 9-1201]), these values were below those with demonstrable HIV-inhibitory activity *in vitro* 12. Median TSP-1 in saliva was 22ng/ml (0-128), and undetectable in CVL. On the other hand, median saliva SLPI levels (1225ng/ml; 64-15,800) exceeded those in CVL (139ng/ml; 2-4930) by nearly 10-fold ($p<0.0001$) and in blood (40ng/ml; 24-100) by 30-fold ($p<0.0001$) (Fig. 1A), suggesting minimal, if any, contribution of these mucosal factors in control of HIV systemically, but potentially limiting infectious virus in mucosal compartments. Highest SLPI levels were found in oral cavity where no infectious virus (IUPM <0.32) was detectable, in contrast to blood, where SLPI was low, and infectious virus was frequently detectable.

Mucosal fluids are also distinct from the systemic compartment by their levels of adaptive immune factors and antibodies that influence viral infectivity. We found that highest levels of both total and HIV-specific IgG and IgA were present in blood (Fig. 1B,C), where HIV RNA and culturable virus were also maximal (Supplemental Table 1), demonstrating their inability to adequately control blood HIV RNA expression or culturable virus. At the mucosal level, differences were also evident in total and specific IgA and IgG. Total IgA was about 16-fold higher in saliva than CVL (Fig. 1B, $p<0.0001$) and HIV gp120-specific IgA was also higher in saliva compared to CVL (median ~40µg/ml vs undetectable in CVL). Potentially, IgA directed to HIV-antigens other than those specific for gp120, measured here, could also be represented in total IgA. By comparison, total IgG was about 3-fold higher in CVL than saliva ($p<0.0001$) (Fig. 1B), but considerably lower than in blood. Similar to levels of total IgG, HIV-specific IgG was represented 2-3-fold more in CVL than saliva ($p<0.0001$) (Fig. 1C). Despite predominance of total IgA in saliva, levels of HIV-

specific IgG were comparable to those of specific IgA, as evident in CVL, suggesting that IgA responses to HIV are quite limited at mucosal sites.

Association of HIV RNA Shedding with Clinical, Virologic and Immunologic Factors

To identify potential factors that could differentially impact HIV RNA shedding in oral (Table 2A) and genital compartments (Table 2B), we examined relationships between local and systemic virologic (HIV RNA, infectious virus) and immunologic factors (CD4 count, TSP, SLPI), as well as co-infections in each compartment. Multiple co-infections that were commonly detected in oral cavity included HHV6 (54%), *Candida* (49%) and EBV (21%), while herpes viruses (CMV, HSV1, HSV2, HHV8) were uncommon (0-3%). In unadjusted models that examined association of these infections as well as SLPI and TSP with oral HIV shedding, we found that antiretroviral therapy (ART), plasma HIV RNA, CD4⁺ counts, quantitative PBMC cultures (IUPM) and EBV were all significantly associated with oral HIV shedding (Table 2A), while the innate molecules SLPI and TSP were not associated with HIV RNA/shedding. When we adjusted for ART and plasma RNA, presence of blood infectious virus (IUPM) retained statistically significant associations with oral shedding, while EBV and CD4 count were no longer associated with shedding of HIV. For each log increase in plasma RNA, odds of oral HIV shedding increased 2.2-fold (95% CI: 1.6-2.9; $p < 0.001$). Furthermore, women receiving HAART had 80% decreased odds (OR: 0.2; 95% CI: 0.1-0.8; $p = 0.02$) of oral HIV RNA compared with untreated women, independent of plasma RNA and CD4. There was no association observed between oral HIV shedding and presence of gingival bleeding, as a measure of inflammation ($n = 28$); however, a statistically significant association was observed for oral shedding with periodontal disease (maximum loss of attachment and pocket depth [$p = 0.01$ and $p = 0.03$, respectively]) and marginally with presence of oral lesions ($n = 30$; $p = 0.08$; data not shown).

Similar to oral cavity, there were multiple co-infections in genital tract, including *Candida* (26%), HPV (39%, including pathogenic strains 16, 18, 31, 33, 35 in 40%) and bacterial vaginosis (18%). CMV and HSV were infrequently detected (1.9% and 8.4%, respectively). As in oral cavity, plasma HIV RNA, CD4⁺ T cells and IUPM were all significantly associated with genital HIV shedding in unadjusted analyses (Table 2B). Additional significant correlates were HPV ($p = 0.01$) and other viral infections ($p = 0.01$). TSP and SLPI were not associated with genital HIV RNA. However, in adjusted analyses, only HIV RNA and infectious virus remained predictors for genital HIV shedding. Multivariate models showed that for each log increase in plasma RNA, odds of genital HIV shedding increased 2.8-fold (95% CI: 2.0-4.0; $p < 0.001$). ART was not associated with genital HIV shedding in this model and 41.2% of women on HAART still had detectable genital HIV RNA.

We next examined the influence of levels of IgG and IgA on HIV RNA shedding in multivariate models (Table 3). Unexpectedly we found that total oral IgA showed a strong positive association with oral HIV RNA levels. Women with the highest tertile of total IgA ($\geq 64.968 \mu\text{g/mL}$) were 37-times (95% CI: 1.8-744.6; $p = 0.02$) more likely to have detectable HIV RNA than women in the lowest tertile ($< 42.728 \mu\text{g/mL}$), adjusting for ART and plasma HIV RNA (Table 3; p -value for trend = 0.03). Adjusting for CD4⁺ counts did not substantially alter these results. Total IgA correlated directly with HIV RNA in unspun saliva (Spearman $r = 0.54$, $p = 0.003$) (Fig. 2A). Level of HIV-specific IgA was also significantly associated with oral HIV shedding (≥ 0.041 vs. $< 0.027 \mu\text{g/mL}$; OR = 9.9; 95% CI: 1.0-97.6; $p = 0.05$) in adjusted analyses, whereas total IgG and HIV-1-specific IgG were not associated with HIV RNA/shedding. Since local mucosal co-infections could influence altered immunoglobulin levels, in addition to distorting immune function, disrupting the epithelial barrier, and recruiting HIV target cells, we also assessed the potential relationship between oral pathogens and immunoglobulin levels. We found a marginal negative

association between specific IgG and HHV6($p=0.08$), with a statistically significant negative association between total IgG and HHV6($p=0.02$).

Analogous to oral cavity, women in the highest tertile of genital IgA($\geq 3.8\mu\text{g/mL}$) were >37 -times(95%CI: 2.6-531.1; $p=0.008$) more likely to have genital HIV shedding as women in the lowest tertile($<2.0915\mu\text{g/mL}$) after adjusting for ART and plasma HIV RNA(Table 3; p -value for trend=0.007). Total IgA, but not HIV-specific IgA in CVL correlated directly with HIV RNA in both cervical swab(Spearman $r=0.45$, $p=0.0008$) and CVL($r=0.44$, $p=0.001$) (Fig. 2B). Genital total IgG(≥ 15.532 vs. $<7.758\mu\text{g/mL}$: OR=4.7; 95%CI: 0.8-28.1; $p=0.09$) showed a similar relationship with viral shedding after adjusting for ART and plasma HIV RNA or after adjusting for ART, plasma RNA and CD4(≥ 15.532 vs. $<7.758\mu\text{g/mL}$: OR=9.1; 95%CI: 1.1-77.7; $p=0.05$), but HIV-specific IgG did not. Thus, despite being a relatively heterogeneous group of HIV⁺ women, it appears that levels of mucosal immunoglobulin, particularly IgA, are linked to local HIV RNA, independent of ART status or plasma virus. Further analyses revealed that in the genital compartment, a marginal positive association between total IgA and SLPI($r=0.27$; $p=0.08$) was evident(data not shown).

Discussion

This study evaluating potential determinants of infectious HIV and HIV RNA/shedding simultaneously in three compartments resulted in a number of notable findings. First, despite HIV RNA being detectable in mucosal fluids, it was rarely culturable, whereas virus was frequently cultured from blood. Second, of several putative innate and adaptive mucosal defense factors examined, only IgA was associated with shedding HIV RNA in the mucosae. However, rather than finding a protective effect, there was a striking increase in the probability of having detectable HIV RNA in the oral or genital tracts in women with highest total IgA levels. Finally, women who were on HAART, albeit less potent than HAART regimens in current use, often had detectable oral or genital HIV RNA, in addition to blood HIV. Nevertheless, among women with undetectable plasma HIV, 8% still had genital shedding and 3% had oral HIV RNA.

Since plasma HIV RNA and infectious virus are prominent determinants of HIV RNA/shedding in both oral and genital compartments, it was surprising that systemic HIV levels were not associated with infectious/culturable virus in the mucosae. While the typically lower levels of mucosal HIV RNA may reflect this disparity in infectious titers, even when plasma HIV RNA was undetectable or at low levels, virus could still be cultured from some blood samples. These data support the notion that there are mucosal-specific factors that compromise the ability of virions to transmit infection, consistent with epidemiologic evidence that rates of mucosal HIV transmission are relatively low per high-risk exposure^{24, 25}. A number of local factors were evaluated as potential determinants of infectious HIV and shedding HIV RNA in mucosal fluids. Although neither SLPI nor TSP-1, two innate mediators, correlated with presence or level of HIV RNA, highest SLPI levels and lack of infectious HIV were found in mucosae. The ability of SLPI to neutralize elastase, associated with HIV shedding², blunt inflammation²⁶, inhibit bacterial and fungal co-infections²⁷, and/or target host cells to inhibit HIV^{10, 11, 13, 28, 29}, may contribute to a resistant, albeit not impenetrable mucosal phenotype. Lower SLPI levels in CVL than saliva are perhaps related to inability to differentiate between active and inactivated SLPI³⁰⁻³¹. In mucosa, SLPI levels are reportedly altered by *Candida albicans*, HSV and/or HIV itself³²⁻³⁴. Increased risk of HIV in HSV2-seropositive individuals^{35, 36} in the context of decreased SLPI³⁴, the association between CVL SLPI titers and perinatal HIV transmission³⁷, and influence of breast milk SLPI on maternal-infant transmission³⁸ underscore the potential significance of this mucosal antimicrobial peptide. Comparative analyses of additional anti-HIV factors, including defensins and chemokines, may reveal molecules that independently,

or in the aggregate, may antagonize infection, particularly if antibodies are inadequately neutralizing.

In this regard, the most significant and consistent association in both genital and oral fluids was between the dominant IgA mucosal antibody isotype and HIV RNA. However, high total IgA levels in the two mucosal compartments (as well as oral HIV-specific IgA) was associated with a higher likelihood of HIV RNA/shedding. Enhanced mucosal total IgA may reflect polyclonal stimulation of IgA-secreting cells by co-infections, chronic activation, or HIV stimulation of mucosal IgA differentiation and secretion³⁹. Total IgA levels may also be influenced by high mucosal levels of SLPI, which regulates IgA through activation induced deaminase(AID)⁴⁰. In chronic HIV infection, polyclonal B cell activation, exhaustion and hypergammaglobulinemia occur in the periphery, and antiviral therapy is associated with a rapid decline in plasma HIV antibodies^{41, 42}, seemingly inconsistent with the mucosal data in these chronically infected women, the majority of whom are on antiviral therapy. Although studies have identified HIV-specific IgA in blood and mucosal secretions that neutralizes HIV in *in vitro* assays^{39, 43-46}, higher IgA in our studies was not related to decreased viral RNA systemically or mucosally. Whether this mirrors the reported HIV-enhancing ability of serum IgA from HIV⁺ individuals⁴⁷, failure of neutralizing antibodies to interfere with HIV transcytosis through epithelial cells⁴⁸ or other mechanism(s) is unclear, but the connection between IgA and enhanced HIV shedding may be relevant in vaccine development⁴⁹ and needs further study. In one recent study, HIV-specific IgA not only did not protect, but was also detected more frequently in breast milk of transmitters⁵⁰. Although our analyses were limited by the cross-sectional design, such observations may relate to the recently described ability of IgA antibodies to bind intact HIV virions and coordinate their basolateral to apical transport across epithelium⁵¹. If this were the case, the increase in mucosal HIV RNA that we observed in the context of elevated total and/or HIV-specific IgA may be related to IgA-mediated HIV excretion into the mucosal lumen, which could also be consistent with reduced infectious virus despite high HIV RNA. While such possibilities await further study, these intriguing findings highlight the need to better understand mucosal innate and adaptive factors that may modify HIV transmission between sexual partners and from mother-to-child and also, confound vaccine development.

Further assessment of suspect local factors affecting mucosal immunoglobulins and HIV demonstrated that inflammatory lesions and the presence of co-pathogens had less influence than anticipated, albeit these analyses may have been hampered by relatively small numbers of exposed women with certain infections. In prior cross-sectional and prospective studies, mucosal co-infections have been associated with enhanced HIV replication^{2, 3}, likely related to breach of mucosal integrity, recruitment of HIV-susceptible targets and/or HIV-infected cells, activation of NFkB and other pathways²⁹. In this regard, CMV, HPV and herpesviruses may directly or indirectly transactivate HIV^{52,53}. EBV⁺ women reportedly have increased risk of perinatal HIV transmission⁵⁴, and in our study, were more likely to have genital or oral HIV RNA in unadjusted analyses. However, when adjusted for plasma RNA levels, this association was lost, suggesting shared mechanisms may influence local viral replication.

No single factor controls HIV shedding and/or replication, but the collective components of the local milieu, including mucosal architecture, co-infections, innate and adaptive immune factors and susceptible target cells with their unique viral tropisms, together with circulating viral burden and systemic immunity, likely determine mucosal expression and infectivity of HIV. Because these studies were performed on samples obtained before the use of more potent HAART regimens currently available, we were able to take advantage of the ability to more readily monitor infectious HIV and HIV RNA in parallel. Though clearly not independent of systemic determinants of viral replication and infectivity, mucosae also

provide an independent source of viral determinants. Characterization of local factors is essential to not only understand HIV transmission and pathogenesis, but also support vaccine development. Since preventing infection is a primary goal to limit the AIDS pandemic, full characterization of endogenous antiviral mechanisms may provide new insights into development of prophylactic and therapeutic approaches against HIV, particularly those involving mucosal tissues, the most common sites of primary HIV transmission.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Immunologic markers in blood and mucosal compartments

A) Median TSP and SLPI levels in saliva, CVL and blood. Observations for both TSP and SLPI were: saliva, n=48; CVL, n=88; and blood, n=114. **B)** Median levels of IgA and IgG in saliva, CVL and blood. Observations for IgA were: saliva, n = 41; CVL, n = 57; and blood, n = 98. Observations for IgG were: saliva, n = 40; CVL, n = 58; and blood, n = 95. **C)** Median levels of HIV-1 specific IgA and IgG in saliva, CVL and blood. Observations for IgA were: saliva, n = 50; CVL, n = 60 (only 3 non-zero results); and blood, n = 67. Observations for IgG were: saliva, n = 50; CVL, n = 60; and blood, n = 65.

Figure 2. IgA and HIV RNA levels

A) Relationship between total IgA in saliva and HIV RNA level in the oral cavity. HIV RNA lower limit of detection (LLD) was $\leq 1.9 \log_{10}$ copies/mL (≤ 80 copies/mL) for both oral swab and unspun saliva. Total IgA correlated directly with HIV RNA level in unspun saliva ($R=0.54$, $p=0.003$). **B)** Relationship between total IgA in CVL and HIV RNA level in genital tract. HIV RNA LLD was $\leq 2.6 \log_{10}$ copies/mL (≤ 400 copies/mL) for cervical swab and $\leq 1.9 \log_{10}$ copies/mL (≤ 80 copies/mL) for CVL. Total IgA in CVL correlated directly with HIV RNA levels in both cervical swab ($R=0.45$, $p=0.0008$) and CVL ($R=0.44$, $p=0.001$).

TABLE 1
Detection of HIV RNA in Plasma, Oral and Genital Mucosa in Presence or Absence of
Antiretroviral Therapy

Compartment	Total (n=106)*	Antiretroviral Therapy		
		None (n=37)	Mono or Combo (n=22)	HAART (n=47)
Blood, genital and oral	24.5% (16.3, 32.7) [†]	35.1% (19.8, 50.5)	18.2% (2.1, 34.3)	19.2% (7.9, 30.4)
Blood only	25.5% (17.2, 33.8)	29.7% (15.0, 44.4)	27.3% (8.7, 45.9)	21.3% (9.6, 33.0)
Blood and genital	14.2% (7.5, 20.8)	10.8% (0.8, 20.8)	4.6% (0, 13.3)	21.3% (9.6, 33.0)
Blood and oral	3.8% (0.1, 7.4)	8.1% (0, 16.9)	0%	2.1% (0, 6.2)
Genital only	1.9% (0, 4.5)	2.7% (0, 7.9)	0%	2.1% (0, 6.2)
Oral only	0.9% (0, 2.8)	0%	4.6% (0, 13.3)	0%
Genital and oral	0%	0%	0%	0%
Undetectable blood, genital, and oral	29.3% (20.6, 37.9)	13.5% (2.5, 24.5)	45.5% (24.6, 66.3)	34.% (20.5, 47.6)

* Women missing HIV RNA in any of the compartments were excluded from analysis. Of the 9 that are missing data, 7 are missing oral, 1 genital and 1 blood.

[†] Values represent % detectable (95% Confidence Interval) and are derived from a log-linear model of the detection of HIV-1 RNA for the 3 compartments (blood, oral cavity, genital tract).

TABLE 2
Presence of HIV RNA in Oral Cavity: Association with Clinical, Virologic and Immunologic Variables (n=108)

	Oral HIV RNA Detected*		Unadjusted		Adjusted for ART and plasma HIV RNA group†	
	n/Total	%	Odds ratio (95% CI)‡	P	Odds ratio (95% CI)	P
Antiretroviral therapy						
None	17/39	43.6%	1		1	
Mono or Combo	5/22	22.7%	0.4 (0.1-1.2)	0.11	0.4 (0.1-1.9)	0.26
HAART	10/47	21.3%	0.3 (0.1-0.9)	0.03	0.2 (0.1-0.8)	0.02
Plasma HIV RNA (copies/mL)						
<400	1/34	2.9%	1		1	
400 to <10,000	8/38	21.1%	8.8 (1.0-74.6)	0.05	6.2 (0.7-55.6)	0.1
≥10,000	23/35	65.7%	63.3 (7.7-520.8)	<0.001§	64.6 (7.5-555.2)	<0.001§
Unknown	0/1	0.0%				
Per log ₁₀ increment	32/107	29.9%	2.1 (1.6-2.8)	<0.001	2.2 (1.6-2.9)	<0.001
CD4+count (cells/mm³)						
<200	11/16	68.8%	10.6 (2.7-42.0)	0.001§	3.4 (0.6-19.7)	0.18
200 to ≤500	15/57	26.3%	1.7 (0.6-5.0)	0.31	0.6 (0.2-2.3)	0.46
>500	6/35	17.1%	1		1	
Quantitative PBMC cultures (IUPM)						
Positive (≥0.3219)	26/58	44.8%	6.3 (2.2-18.4)	0.001	5.5 (1.9-16.3)	0.002
Negative (<0.3219)	5/44	11.4%	1		1	
Unknown	1/6	16.7%				
TSP (ng/mL)						
<7	5/16	31.3%	1		1	
7 to <35.5	7/15	46.7%	1.9 (0.4-8.3)	0.38	4.0 (0.4-36.4)	0.22
≥2425	3/16	18.8%	0.5 (0.1-2.6)	0.42	0.5 (0.05-5.3)	0.56
Unknown	17/61	27.9%				
SLPI (ng/mL)						

	Oral HIV RNA Detected*		Unadjusted		Adjusted for ART and plasma HIV RNA group [‡]	
	n/Total	%	Odds ratio (95% CI) [‡]	P	Odds ratio (95% CI)	P
<893.5	4/16	25.0%	1		1	
	6/15	40.0%	2.0 (0.4-9.3)	0.89	0.8 (0.1-7.4)	0.86
	5/16	31.3%	1.4 (0.3-6.4)	0.7	1.2 (0.1-10.3)	0.86
	17/61	27.9%				
Candidiasis						
Present	18/52	34.6%	1.6 (0.7-3.6)	0.3	1.1 (0.4-3.1)	0.89
Absent	14/55	25.5%	1		1	
Unknown	0/1	0.0%				
Epstein-Barr virus						
Present	11/22	50.0%	3.3 (1.2-8.7)	0.02	1.9 (0.5-6.8)	0.32
Absent	19/81	23.5%	1		1	
Unknown	2/3	40.0%				
Human herpesvirus 6						
Present	15/56	26.8%	0.8 (0.3-1.8)	0.57	1.5 (0.5-4.4)	0.5
Absent	15/47	31.9%	1		1	
Unknown	2/3	40.0%				
Other viral infections (CMV, HHV8, HSV)						
Present	1/6	16.7%	0.5 (0.1-4.9)	0.59	0.3 (0.02-3.8)	0.32
Absent	22/82	26.8%	1		1	
Unknown	9/20	45.0%				

ART=antiretroviral therapy; PBMC=peripheral blood mononuclear cells; IUPM=infectious units per million cells

[‡] Odds ratios, 95% confidence intervals and p-values estimated from logistic regression.

* HIV RNA was present if HIV RNA was above the lower limit of detection in either the oral swab or saliva assay. Presence of HIV RNA is unknown if both assays were not completed.

[‡] Antiretroviral therapy model adjusted for plasma HIV RNA only. Plasma HIV RNA and Quantitative PBMC culture models adjusted for ART only.

[§] P-value from trend test <0.05.

TABLE 3
Presence of HIV RNA in Genital Tract: Association with Clinical, Virologic and Immunologic Variables (n=114)

	Genital HIV RNA detected*		Unadjusted		Adjusted for ART and Plasma HIV RNA group†	
	n/Total	%	Odds ratio (95% CI)‡	P	Odds ratio (95% CI)	P
Antiretroviral therapy						
None	18/38	47.4%	1		1	
Mono or Combo	7/25	28.0%	0.4 (0.1-1.3)	0.13	0.5 (0.1-1.9)	0.28
HAART	21/51	41.2%	0.8 (0.3-1.8)	0.56	1.0 (0.3-3.1)	0.97
Plasma HIV RNA (copies/mL)						
<400	3/37	8.1%	1		1	
400 to <10,000	13/40	32.5%	5.5 (1.4-21.1)	0.01	5.1 (1.3-20.7)	0.02
≥10,000	30/36	83.3%	56.7 (13.0-246.5)	<0.001§	57.1 (12.8-255.4)	<0.001§
Unknown	0/1	0.0%				
Per log ₁₀ increment	46/113	40.7%	2.8 (2.0-4.0)	<0.001	2.8 (2.0-4.0)	<0.001
CD4+ count (cells/mm³)						
<200	13/16	81.3%	11.7 (2.7-49.9)	0.001§	3.0 (0.5-16.8)	0.22
200 to ≤500	23/61	37.7%	1.6 (0.7-4.0)	0.28	0.8 (0.3-2.6)	0.75
≥500	10/37	27.0%	1		1	
Quantitative PBMC cultures (IUPM)						
Positive (≥0.3219)	37/59	62.7%	8.6 (3.4-21.7)	<0.001	8.8 (3.4-22.8)	<0.001
Negative (<0.3219)	8/49	16.3%	1		1	
Unknown	1/6	16.7%				
SLPI (ng/mL)						
<74.94	12/29	41.4%	1		1	
74.94 to <196	10/29	34.5%	0.7 (0.3-2.2)	0.59	1.6 (0.4-6.8)	0.51
≥196	13/30	43.3%	1.1 (0.4-3.0)	0.88	1.2 (0.3-5.1)	0.76
Unknown	11/26	42.3%				
Candidiasis						

	Genital HIV RNA detected*		Unadjusted		Adjusted for ART and Plasma HIV RNA group [†]	
	n/Total	%	Odds ratio (95% CI) [‡]	P	Odds ratio (95% CI)	P
Present by culture	14/29	48.3%	1.5 (0.6-3.6)	0.34	1.1 (0.3-3.4)	0.93
Absent by culture	32/84	38.1%	1		1	
Unknown	0/1	0.0%				
Bacterial vaginosis						
Present	10/21	47.6%	1.4 (0.6-3.7)	0.45	1.4 (0.4-4.7)	0.62
Absent	36/93	38.7%	1		1	
Human papillomavirus, any type						
Present	21/42	50.0%	1.8 (0.8-3.9)	0.15	0.8 (0.3-2.4)	0.66
Absent	24/67	35.8%	1		1	
Unknown	1/5	20.0%				
Human papillomavirus, types 16, 18, 31, 33, 35						
Present	12/17	70.6%	4.3 (1.4-13.2)	0.01	2.6 (0.7-10.4)	0.18
Absent	33/92	35.9%	1		1	
Unknown	1/5	20.0%				
Other viral infections (CMV, HSV)						
Present	9/11	81.8%	8.0 (1.6-39.1)	0.01	1.9 (0.3-11.6)	0.48
Absent	37/103	35.9%	1		1	

ART=antiretroviral therapy; PBMC=peripheral blood mononuclear cells; IUPM=infectious units per million cells;

[‡] Odds ratios, 95% confidence intervals and p-values estimated from logistic regression.

* HIV RNA was present if HIV RNA was above the lower limit of detection in either the cervical swab or CVL assay. Presence of HIV RNA is unknown if both assays were not completed.

[†] Antiretroviral therapy model adjusted for plasma HIV RNA only. Plasma HIV RNA and Quantitative PBMC culture models adjusted for ART only.

[§] P-value from trend test <0.05.

TABLE 4
Presence of HIV RNA in Oral Cavity and Genital Tract: Association with Immunoglobulin (n=108)

	Oral HIV RNA Detected*		Unadjusted		Adjusted for ART and plasma HIV RNA group	
	n/total	%	Odds ratio (95% CI) [‡]	P	Odds ratio (95% CI)	P
Total IgA (µg/mL)						
<42.728	2/13	15.4%	1		1	
42.728 to <64.968	4/14	28.6%	2.2 (0.3-14.7)	0.42	5.2 (0.2-106.7)	0.29
≥64.968	8/13	61.5%	8.8 (1.3-57.4)	0.02§	37.0 (1.8-744.6)	0.02§
Unknown	18/68	26.5%				
Total IgG (µg/mL)						
<2.609	5/13	38.5%	1		1	
2.609 to <5.631	2/12	16.7%	0.3 (0.05-2.1)	0.24	0.4 (0.03-4.5)	0.44
≥5.631	7/14	50.0%	1.6 (0.3-7.4)	0.55	2.9 (0.3-26.9)	0.34
Unknown	18/69					
HIV-Specific IgA (µg/ml)						
<0.027	4/16	25.0%	1		1	
0.027 to <0.041	4/15	26.7%	1.1 (0.2-5.5)	0.92	1.1 (0.1-10.9)	0.91
≥0.041	8/18	44.4%	2.4 (0.6-10.4)	0.24	9.9 (1.0-97.6)	0.05
Unknown	16/59	27.1%				
HIV-Specific IgG (µg/ml)						
<0.016	5/16	31.3%	1		1	
0.016 to <0.046	4/16	25.0%	0.7 (0.2-3.5)	0.7	0.3 (0.02-3.3)	0.29
≥0.046	7/17	41.2%	1.5 (0.4-6.4)	0.56	1.2 (0.1-11.7)	0.9
Unknown	16/59	27.1%				
Genital HIV RNA detected[¶]					Adjusted for ART and Plasma HIV RNA group	
	n/total	%	Unadjusted		Odds ratio (95% CI)	
				P		P
Total IgA (µg/ml)						
<2.0915	4/19	21.1%	1.0		1.0	

	Oral HIV RNA Detected*		Unadjusted		Adjusted for ART and plasma HIV RNA group	
	n/total	%	Odds ratio (95% CI) [‡]	P	Odds ratio (95% CI)	P
2.0915 to <3.837	8/19	42.1%	2.7 (0.7-11.4)	0.17	7.5 (0.7-81.7)	0.10
≥3.837	13/18	72.2%	9.8 (2.2-44.1)	0.003 [§]	37.2 (2.6-531.1)	0.008 [§]
Unknown	21/58	36.2%				
Total IgG (µg/ml)						
<7.758	8/19	42.1%	1		1	
7.758 to <15.532	5/19	26.3%	0.5 (0.1-1.9)	0.31	1.0 (0.2-6.2)	0.97
≥15.532	12/19	63.2%	2.4 (0.6-8.7)	0.2	4.7 (0.8-28.1)	0.09
Unknown	21/57	36.8%				
HIV-Specific IgA (µg/ml)						
<0.02	23/56	41.1%	n/a		n/a	
≥0.02	3/3	100.0%				
Unknown	20/55	36.4%				
HIV-Specific IgG (µg/ml)						
<0.0425	12/20	60.0%	1		1	
0.0425 to <0.1245	5/19	26.3%	0.2 (0.1-0.9)	0.04	0.2 (0.04-1.2)	0.07
≥0.1245	9/20	45.0%	0.5 (0.2-1.9)	0.34	0.7 (0.1-3.6)	0.67
Unknown	20/55	36.4%				

ART=antiretroviral therapy; PBMC=peripheral blood mononuclear cells; IUPM=infectious units per million cells;

[‡] Odds ratios, 95% confidence intervals and p-values estimated from logistic regression.

* HIV RNA was present if HIV RNA was above the lower limit of detection in either the oral swab or saliva assay. Presence of HIV RNA is unknown if both assays were not completed.

[†] HIV RNA was present if HIV RNA was above the lower limit of detection in either the cervical or CVL assay. Presence of HIV RNA is unknown if both assays were not completed.

[§] P-value from trend test <0.05.