

Coinfection with Herpes Simplex Virus Type 2 Is Associated with Reduced HIV-Specific T Cell Responses and Systemic Immune Activation

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Background. Chronic coinfection with herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus (HIV) has been associated with an increased HIV viral load and more rapid disease progression, perhaps related to HSV-2-associated alterations in host immunity.

Methods. Studies were nested within (1) a cross-sectional study of men coinfecting with HIV and HSV-2 and (2) women not infected with HIV, both before and after HSV-2 acquisition. HSV-2 infection status was determined by ELISA. HIV-specific CD8⁺ T cell epitopes were mapped, and proliferation of HIV-specific cells was also assessed. Systemic inflammatory and regulatory T cell populations were assayed by flow cytometry.

Results. The breadth of both the HIV-specific CD8⁺ T cell interferon- γ and proliferative responses was reduced in participants coinfecting with HIV and HSV-2, independent of the HIV plasma viral load and CD4⁺ T cell count, and the magnitude of the responses was also reduced. HSV-2 infection in this group was associated with increased T cell CD38 expression but not with differences in the proportion of CD4⁺ FoxP3⁺ regulatory T cells. However, in women not infected with HIV, acquisition of HSV-2 was associated with an increase in the proportion of regulatory T cells.

Conclusions. HSV-2 coinfection was associated with reduced HIV-specific T cell responses and systemic inflammation. The immune effects of HSV-2 may underlie the negative impact that this coinfection has on the clinical course of HIV infection.

Infection with herpes simplex virus type 2 (HSV-2) has been shown to affect the acquisition and subsequent course of HIV-1 (hereafter, "HIV") in several ways. Chronic HSV-2 infection increases HIV acquisition rates approximately 3-fold for both men and women [1] and up to 6-fold for high-risk female sex workers [2]. In

sub-Saharan Africa, where the HIV pandemic has hit hardest, >60% of the general population may be infected by HSV-2 [3]. At this prevalence, it has been estimated that as many as half of the incident cases of HIV infection can be directly attributed to infection with HSV-2 [4].

HSV-2 coinfection is not only an important risk factor for HIV acquisition, but it also affects the subsequent course of HIV disease and secondary transmission of HIV infection [5]. Infection with HSV-2 has been associated with higher HIV viral load during acute infection [6], as has HSV-2 reactivation during chronic HIV infection [7, 8]. HSV-2 suppressive treatment with acyclovir for individuals coinfecting with HIV and HSV-2 reduced both genital HSV-2 reactivation and genital HIV shedding [9]. In addition, acyclovir therapy reduced the HIV blood viral load by >0.5 log₁₀ copies/mL, a reduction that would be expected to affect the clinical course of HIV significantly and may explain the survival benefit

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provided by acyclovir for individuals with advanced HIV infection [10]. These findings clearly demonstrate that HSV-2 coinfection has a direct, deleterious effect on host control of HIV, and clinical trials are currently examining the potential for HSV-2-suppressive therapy to delay the progression of HIV disease.

It is not known how coinfection with HSV-2 affects host immune control of HIV. HSV-2-associated increases in HIV susceptibility may be related to genital macroulceration or microulceration during reactivation [11] and/or to the increased number of HIV target cells present in the genital mucosa (dendritic cells expressing DC-SIGN [dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin] and activated CD4⁺ T cells), even in the absence of HSV-2 reactivation [12]. If HSV-2 were to induce systemic immune activation in addition to these mucosal changes, this could negatively affect HIV pathogenesis in several ways. HIV replication is enhanced in activated CD4⁺ T cells [13], which can lead to increases in HIV viral load and possibly to a more profound depletion of CD4⁺ T helper cells. In particular, expression of the activation marker CD38 by T cells in HIV-infected individuals has been associated with CD4⁺ depletion [14] and may be as good as or better than total CD4⁺ T cell count as a prognostic marker for disease progression [15, 16].

Alternatively, chronic immune activation could induce a regulatory T cell response, with subsequent impairment in T cell responses to other copathogens, including HIV. Animal models have demonstrated that immune control of HSV-2 is enhanced if natural regulatory T cells are depleted before HSV-2 infection, and it has been hypothesized that regulatory T cells induced by acute HSV-2 infection may impair the immune response to other infectious challenges [17]. In the setting of HIV infection, regulatory T cells' proportion and/or number may be increased, particularly in advanced disease [18], and these cells suppress HIV-specific cytolytic T cell function, perhaps impairing the ability to control HIV replication *in vivo* [19].

On the basis of these prior observations, we hypothesized that coinfection with HSV-2 would be associated with systemic immune activation, increased regulatory T cell activity, and impaired HIV-specific T cell responses in HIV-infected individuals. We tested these hypotheses in well-established cohorts of HIV-infected participants and participants not infected with HIV, from Canada and Kenya, respectively.

METHODS

Research participants. HIV-infected men were recruited from a cohort in Toronto, Ontario, of men who have sex with men. All of the men had been HIV infected for ≥ 1 year, were antiretroviral therapy naive, and were not expected to require antiretroviral therapy within the next year. Studies of the impact of acute HSV-2 infection were nested within a clinical trial of HIV and sexually transmitted infection prevention performed

between 1998 and 2002 that involved high-risk female sex workers not infected with HIV, who were from the Kibera slum of Nairobi, Kenya [2]. All participants provided informed, written consent; ethical approval for these studies, including specific approval for nested immunologic studies within the Kenyan clinical trial, were obtained through the research ethics board of the appropriate collaborating institutions (the Universities of Toronto, Manitoba, and Nairobi).

Sample processing and diagnostic procedures. HSV-2 serology testing was performed on cryopreserved plasma samples by use of an HSV-2 IgG enzyme immunoassay (Kalon Biological) [2]. Peripheral blood mononuclear cells (PBMCs) were isolated from blood collected into acid citrate dextran solution A; BD Biosciences), by use of density gradient centrifugation over ficoll-hypaque solution (Ficoll-Paque Plus; Amersham Biosciences). Blood was transported to the laboratory within 3 h of collection, layered onto ficoll-hypaque, and subsequently centrifuged at 500 g for 25 min without brakes, counted, and washed twice in RPMI 1640 with 10% heat-inactivated fetal bovine serum (FBS; Sigma), 100 U/mL penicillin, 100 mg/mL streptomycin, and 1 \times GlutaMAX-1 (Gibco). PBMCs were then cryopreserved in fetal bovine serum with 10% DMSO and stored at -150°C until use. Blood plasma was flash frozen at -80°C . PBMCs and plasma samples were collected at a single time point from the Toronto cohort of men who have sex with men, and samples were collected and stored every 3 months during the clinical trial. Blood HIV RNA viral load was measured using the Versant HIV-1 RNA 3.0 assay (bDNA; Bayer Diagnostics).

Assessment of CD8⁺ T cell interferon (IFN)- γ responses by ELISpot assay. HIV-specific CD8⁺ T cell responses were mapped in blood by use of an IFN- γ ELISpot assay, as described elsewhere [20]. Fresh PBMCs were incubated at 1×10^5 per well with a matrix of 756 15-mer peptides, overlapping by 11 aa, spanning the entire clade B HIV-1 genome (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health). Each peptide appeared uniquely in 2 separate matrix pools, at a final working concentration of 2 $\mu\text{mol/L}$. All responses detected by use of the matrix pools were confirmed in cryopreserved PBMCs by use of individual 15-mer peptides, and they were confirmed to be CD8⁺ T cell mediated by intracellular cytokine staining. Response frequencies were calculated with an automated ELISpot counter (Cellular Technology), and a positive response was defined as an HIV peptide-specific response that was (1) ≥ 2 -fold higher than background (PBMCs in tissue culture medium alone), and (2) ≥ 100 spot-forming units (sfu) per million cells [21].

HIV-specific proliferation assays. Seven million PBMCs were resuspended in 1 mL of medium and incubated with carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes) at a final concentration of 1.5 $\mu\text{mol/L}$ at room temperature for 5 min. CFSE-labeled cells were then washed 3 times and resus-

pended in a sterile 24-well tissue culture plate at 37°C in an atmosphere of 5% carbon dioxide for 5 days, either in medium alone, with staphylococcal enterotoxin B (Sigma Aldrich Canada) at 2 µg/mL, or with HIV peptide pools. Four separate HIV gene pools were made up, which spanned HIV-1 Gag, Pol, Env, and a mixed pool of accessory gene peptides (Rev, Nef, Tat, Vif, Vpr, and Vpu), to a final concentration of 0.1 µg/mL for each peptide. A single pool of peptides spanning the entire HIV-1 genome was also tested, with each peptide at a final concentration of 0.01 µg/mL. On day 5, cells were harvested and stained with CD3 phycoerythrin, CD4 peridinin-chlorophyll protein, and CD8 allophycocyanin. Flow cytometric acquisition and analysis was performed using a FACSCalibur flow cytometer (BD Pharmingen) with FlowJo software (version 7.2.2; Tree Star), after gating on CD3⁺ T lymphocytes. A positive response was defined as proliferation in the HIV peptide well >0.05% of gated cells and exceeding background levels of proliferation by ≥3-fold [22].

Peripheral blood immune cell phenotyping. Cryopreserved PBMCs were thawed and rested for 6 h at 37°C in an atmosphere of 5% carbon dioxide before staining. One aliquot was then stained for T cell activation markers CD69, CD38, CD4, and CD3 (BD Pharmingen), and the other for regulatory T cell populations CD25, CD4, CD3, and FoxP3 (BD Pharmingen). Appropriate isotype control antibodies were used in parallel. Samples were obtained and analyzed with a FACSCalibur flow cytometer. All flow cytometry was performed by research personnel blinded to the participant's HSV-2 infection status.

Statistical analysis. All analyses were performed with SPSS software (version 11.0; SPSS). The associations of continuous variables with HSV-2 infection status were determined by using a Mann-Whitney *U* nonparametric test. Comparisons between discrete variables were performed using the χ^2 test with calculations of likelihood ratios. Bivariate correlations were calculated using the Pearson 2-tailed test.

RESULTS

HIV-infected study participants. The association of chronic HSV-2 infection with alterations in HIV-specific immunity and systemic immune activation was examined in 28 chronically HIV-infected, therapy-naive men with a median CD4⁺ T cell count of 555 cells/mm³ (range, 120–1260 cells/mm³) and a median blood plasma viral load of 19,795 copies/mL plasma (range, <50 to 401,448 copies/mL). All participants had been HIV infected for ≥6 months. No participant was receiving therapy for HSV-2 infection or had active anal and/or genital lesions at the time of blood collection. The date of HSV-2 acquisition was not known.

HSV-2 infection and HIV-specific CD8⁺ T cell IFN- γ responses. HIV-specific CD8⁺ T cell IFN- γ responses were mapped in HIV-infected men with a matrix of clade B consensus

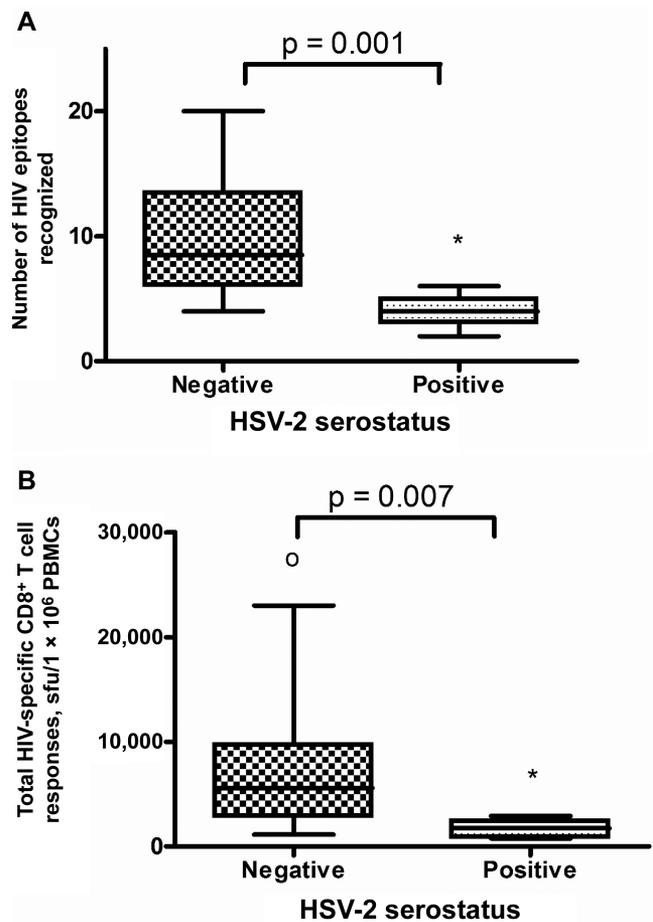


Figure 1. Coinfection with HIV and herpes simplex type 2 (HSV-2) was associated with reduced HIV-specific CD8⁺ T cell responses in therapy-naive men. Clade B HIV-specific CD8⁺ T cell responses were mapped in peripheral blood mononuclear cells (PBMCs) obtained from HIV-infected, therapy-naive men by use of the interferon- γ ELISpot assay. Both the mean number of HIV epitopes recognized (A) and the total HIV-specific response (B) were reduced in HIV/HSV-2-coinfected (right boxes) compared with HIV-monoinfected (left boxes) participants. Box plots show the response interquartile range (IQR) (boxes), the lower quartile (lower whiskers), median (lines within boxes), and the upper quartile (upper whiskers). Asterisks and circle represent outliers >1.5 times outside the IQR.

overlapping peptides that spanned the HIV genome, using the ELISpot assay. All responses were confirmed by IFN- γ intracellular cytokine staining to be CD8⁺ mediated, and all T cell assays were performed by research personnel blinded to the participant's HSV-2 infection status. The mean number of HIV epitopes recognized in participants who were coinfecting with HIV and HSV-2 was significantly lower than that in participants with HIV monoinfection (4.6 vs. 9.9 epitopes; $P = .001$) (figure 1A). The magnitude of the total HIV-specific IFN- γ response, defined as the sum of all epitope responses, was also lower in participants coinfecting with HIV and HSV-2 (2611 vs. 8267 sfu/1 × 10⁶ PBMCs; $P = .007$) (figure 1B), and there was a trend

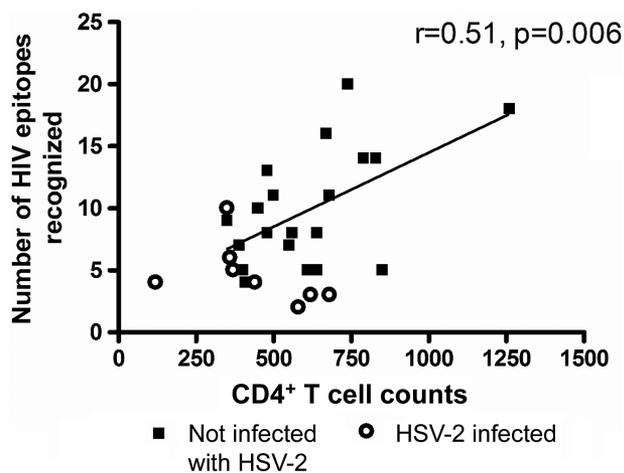


Figure 2. Association between breadth of the CD8⁺ T cell response and immune status. The breadth of the HIV-specific CD8⁺ T cell response was negatively correlated with peripheral blood CD4⁺ T cell counts. Findings are shown for HIV-monoinfected participants (squares) and participants coinfecting with HIV and herpes simplex virus type 2 (HSV-2) (circles).

toward a reduced magnitude of the immunodominant epitope response (1229 vs. 2482 sfu/1 × 10⁶ PBMCs; *P* = .07).

HSV-2–coinfected men had a plasma viral load similar to that of men with HIV monoinfection (4.1 vs. 4.2 log₁₀ RNA copies/mL; *P* = .6) but tended to have a lower CD4⁺ T cell count (440 vs. 614 cells/mm³; *P* = .06). Furthermore, the CD4⁺ T cell count was positively correlated with the number of HIV epitopes recognized (*r*² = 0.51; *P* = .006) (figure 2) and the total magnitude of the HIV-specific response (*r*² = 0.32; *P* = .1) but was negatively correlated with the HIV plasma viral load (*r*² = −0.46; *P* = .01). In a subgroup analysis that categorized participants on the basis of HSV-2 infection status, the association between CD4⁺ T cell count and the number of HIV epitopes recognized remained significant for participants not infected with HSV-2 (*n* = 20; *r*² = 0.55; *P* = .01) but was lost in the smaller HSV-2–infected subgroup (*n* = 8; *r*² = −0.46; *P* = .3). To ensure that differences in HIV disease stage and/or blood viral load did not account for the associations observed between HSV-2 infection and HIV-specific immune responses, a multivariable logistic regression model was established, which incorporated HIV plasma viral load, CD4⁺ T cell count, and HIV-specific CD8⁺ response breadth or magnitude. The association between reduced HIV-specific CD8⁺ response breadth and HSV-2 infection status remained significant in the multivariable model, independent of HIV plasma viral load and CD4⁺ T cell count (*P* = .05) (table 1).

HIV-specific proliferation in participants coinfecting with HIV and HSV-2. In a subset of 12 participants with cryopreserved PBMCs from the same study visit, we examined HIV-specific proliferation in response to overlapping, pooled peptides that spanned consensus clade B HIV Gag, Pol, Env, and a

mixed pool of accessory gene peptides (Rev, Nef, Tat, Vif, Vpr and Vpu). HIV-specific proliferation in response to the peptide pool that spanned the entire HIV genome was demonstrated in 8 (67%) of 12 participants. The strength of the CD8⁺ T cell proliferative response was negatively correlated with the plasma viral load (*r*² = −0.66; *P* = .02), although this association was largely driven by 1 participant with a strong HIV-specific proliferative response (>11%) and an undetectable plasma viral load; statistical significance was lost when this individual was excluded. CD8⁺ T cells from HSV-2–coinfected participants proliferated in response to fewer individual peptide pools (2.3 vs. 3.1 pools; *P* = .01), and the association of HSV-2 infection with a reduced breadth of HIV-specific CD8⁺ T cell proliferation remained strong in a multivariable model that incorporated HIV plasma viral load and CD4⁺ T cell count (*P* = .02) (table 1). In addition, both the magnitude of the total CD8⁺ T cell proliferative response (sum of the CD8⁺ T cell proliferative responses to each of the 4 HIV gene pools, 36.6 for coinfecting vs. 92.9 for monoinfected participants; *P* = .1) and Gag-specific CD8⁺ T cell proliferation (0.05% for coinfecting participants vs. 0.7% for monoinfected participants; *P* = .1) tended to be lower in HSV-2–coinfected participants. Therefore, despite the small number of participants in this substudy, HSV-2 coinfection was also associated with reduced HIV-specific CD8⁺ T cell proliferative responses.

Systemic inflammation and regulatory T cells in HIV-infected men. To test the hypothesis that impaired priming and/or function of adaptive cellular immune responses in HSV-2–infected participants might be due to chronic inflammation and subsequent induction of systemic regulatory T cells, the expression of CD69 and CD38 by peripheral blood T cells was measured (figure 3A), as was the expression of CD25 and FoxP3 (figure 3A). The association of HSV-2 infection with these parameters was then examined in a regression model that included both CD4⁺ T cell count and plasma HIV viral load. Prevalent

Table 1. Multivariable associations with the breadth of the HIV-specific CD8⁺ T cell response.

Response, variable	<i>t</i>	<i>P</i> ^a
IFN-γ release in CD8⁺ T cells^b		
HIV RNA viral load	0.6	.95
CD4 ⁺ T cell count	1.8	.08
HSV-2 infection status	−2.1	.05
CD8⁺ T cell proliferation		
HIV RNA viral load	−0.3	.75
CD4 ⁺ T cell count	−1.1	.31
HSV-2 infection status	−3.0	.02

NOTE. HIV RNA viral load was measured in log₁₀copies/mL; CD4⁺ T cell count, in cells/mm³. HSV-2, herpes simplex virus type 2; IFN, interferon.

^a Determined by logistic regression.

^b Measured by ELISpot assay.

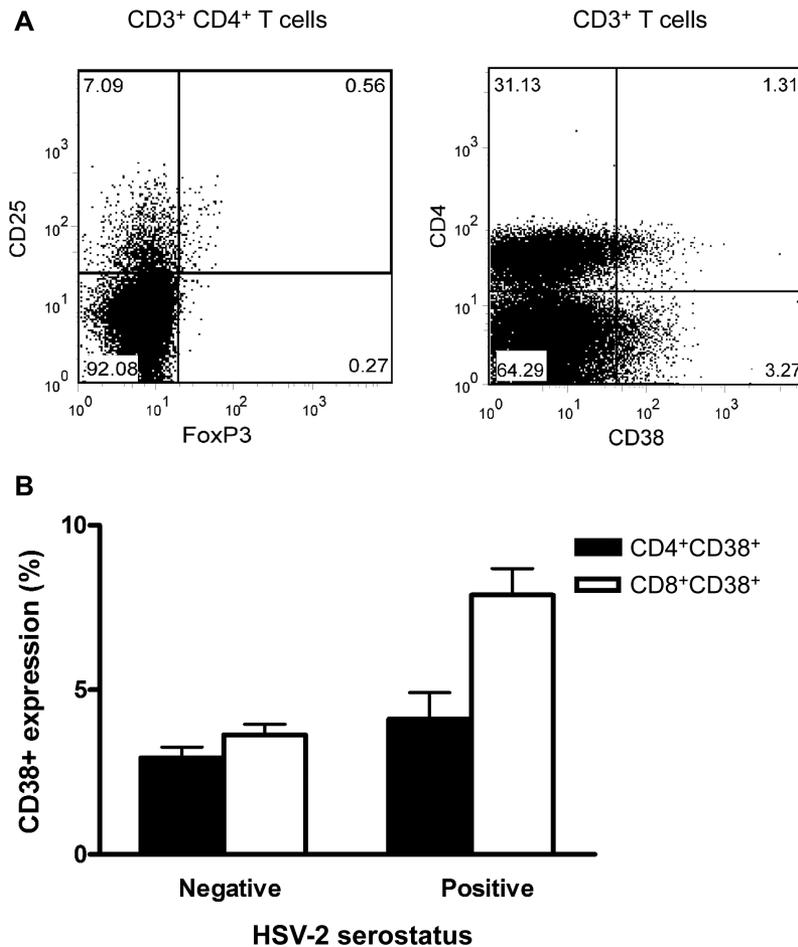


Figure 3. Association of herpes simplex virus type 2 (HSV-2) infection with the expression of activation and regulatory markers on CD4⁺ and CD8⁺ T cells. *A*, Representative fluorescence-activated cell sorter plots showing levels of CD25 and FoxP3 expression by CD4⁺ T cells (*left panel*) and CD38 expression by CD3⁺ T cells (*right panel*). *B*, Expression of CD38, a marker of chronic cell activation, was significantly increased on both CD4⁺ (*black bars*) and CD8⁺ (*white bars*) T cells from men coinfecting with HIV and HSV-2.

HSV-2 infection was independently associated with increased CD38 expression by both CD4⁺ T cells (4.1% vs. 2.9%; $P = .02$) (figure 3*B*) and CD8⁺ T cells (7.9% vs. 3.6%; $P = .002$) (figure 3*B*), but no differences in CD69 expression were observed (data not shown). However, HSV-2 was not associated with differences in the proportion of regulatory CD4⁺ T cells, defined either as CD4⁺ T cells expressing FoxP3 (0.7% vs. 0.9%; $P = .4$) or as coexpression of CD25 and FoxP3 (0.3% vs. 0.4%; $P = .3$) (figure 4).

Immune associations of incident HSV-2 infection in women not infected with HIV. Because HIV infection has important effects on regulatory T cell populations [18, 23–25], a cross-sectional study of HIV-infected participants cannot determine whether the HIV infection or the HSV-2 infection is driving changes in systemic inflammation and/or regulatory T cells. Therefore, the effect of HSV-2 infection status on these same blood immune cell populations was examined in susceptible individuals who were not infected with HIV, using cryopreserved PBMC samples collected from 10 HIV-negative Kenyan

women before and after HSV-2 acquisition. Participants were enrolled from a study of the immune and clinical correlates of protection against HIV acquisition, which has been described elsewhere [2, 26].

The acquisition of HSV-2 was associated with an increase in the expression of FoxP3 by blood CD4⁺ T cells (1.33% after vs. 0.86% before HSV-2 infection; $P = .05$) and with a trend to increased CD4⁺ T cell coexpression of FoxP3 and CD25 (0.91% after vs. 0.72% before HSV-2 infection; $P = .07$). There were no significant differences in the expression of CD38 by CD4⁺ or CD8⁺ T cell populations (data not shown).

DISCUSSION

In these studies, we demonstrate that coinfection with HIV and HSV-2 was associated with narrower and weaker HIV-specific IFN- γ and proliferative T cell responses and with increased systemic T cell immune activation, as measured by CD38 expression. These differences were seen during chronic HSV-2 infec-

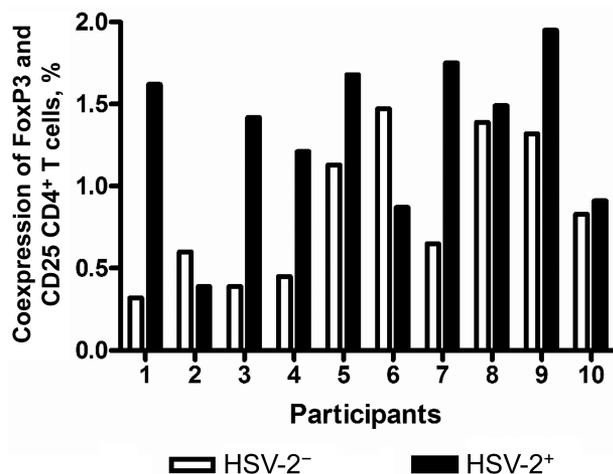


Figure 4. Increases in regulatory T cell populations after herpes simplex virus type 2 (HSV-2) infection in Kenyan women not infected with HIV. The percentage of CD4⁺ T cells expressing FoxP3 and CD25 before (white bars) and after (black bars) HSV-2 acquisition are shown for Kenyan women not infected with HIV.

tion, in the absence of clinically apparent HSV-2 reactivation. Although regulatory T cell numbers did not vary with HSV-2 status in HIV-infected participants, HSV-2 acquisition in individuals not infected with HIV was associated with increases in regulatory T cell numbers.

The rates of disease progression after HIV acquisition are extremely variable, and the factors determining this heterogeneity are poorly understood. Two strong, independent prognostic factors are the plasma HIV viral load set point [27–29] and the degree of systemic immune activation, as indicated by CD38 expression on CD4⁺ and CD8⁺ T cells [15, 16, 30–33]. Chronic HSV-2 infection not only predisposes individuals to the acquisition of HIV [3, 34]—it also has a number of negative effects on HIV immunopathogenesis and disease progression. Chronic infection with or reactivation of HSV-2 has been associated with a higher HIV plasma viral load in several [6–8] but not all [35] studies. HSV-2 can activate HIV transcription directly and also activates host Toll-like receptors, which have been linked to systemic inflammation and HIV progression [36]. Importantly, HSV-2-specific therapy has been associated with a reduction in the HIV plasma viral load of approximately 0.5 log₁₀ copies/mL [9] and with delayed HIV disease progression [10].

Virus-specific CD8⁺ T cell responses are critical to host control of HIV after infection [37], and responses directed against HIV Gag, in particular, have been associated with reduced HIV viral load [38]. The polyfunctionality of the CD8⁺ T cell response may be important in HIV immune control [39], particularly the ability of HIV-specific CD8⁺ T cells to proliferate [40]. In this study, we saw no association between IFN- γ responses and HIV viral load. Although there was a negative correlation between HIV-specific CD8⁺ T cell proliferation and HIV viral load, this was primarily driven by 1 participant with a strong

proliferative response and a low viral load, and the correlation was not significant when this outlier was excluded. Therefore, although HSV-2 infection was clearly associated with reduced CD8⁺ T cell responses, this reduction was not clearly associated with an increased HIV viral load.

Systemic inflammation due to an ineffective HIV-specific cellular immune response may also contribute to immunopathogenesis [41, 42]. Regulatory T cells have potent suppressive activity against the cytolytic function of HIV-specific CD8⁺ T cells and may also suppress HIV-specific CD4⁺ T cells [19, 23, 25]. In addition, HSV-2 impaired the priming of SIV-specific CD8⁺ T cell responses in macaques [43]. Therefore, the induction of regulatory T cells during HIV infection (or other chronic infection) might have 2 dichotomous outcomes [44]. On the one hand, they may impair the priming and/or function of HIV-specific immunity, resulting in inadequate pathogen control and increased tissue damage; on the other, they may limit collateral tissue damage due to unchecked immune activation. In vivo, the effects of regulatory T cells on HIV disease progression have been unclear. Some groups have associated them with higher CD4⁺ T cell counts [18] and reduced systemic immune activation [45], whereas others have found the opposite [46].

In our participants, HSV-2 infection was associated with reduced HIV-specific T cell responses in the absence of differences in regulatory T cell frequency or HIV plasma viral load. It is possible that HSV-2-associated alterations were masked by the impact of subsequent HIV infection but had been present at the time of HIV acquisition and were responsible for the differences observed. This hypothesis was supported by the changes in regulatory T cell frequency associated with HSV-2 acquisition in participants not infected with HIV, although participant numbers in this substudy were small, and this observation requires confirmation. An alternate hypothesis is that the HSV-2-associated systemic immune activation observed in HIV-infected participants was itself responsible for impaired priming and/or function of HIV-specific CD8⁺ T cells, because CD38 expression by CD8⁺ T cells has been associated with impaired function [47, 48].

A possible concern is the use of cryopreserved lymphocytes for phenotypic studies and assessment of HIV-specific T cell responses, with the potential loss of activated and/or antigen-specific T cells during the freeze-thaw process. However, a strong correlation has been described between T cell responses assayed using fresh samples and those assayed with frozen samples [49], and if it had any effect a loss in function should have reduced our ability to detect HSV-2-associated differences. Because we observed several interrelated HSV-2-associated differences in immune function and phenotype despite a relatively modest sample size, we believe that the association observed between chronic HSV-2 infection and these parameters was robust.

Overall, coinfection with HSV-2 in this cohort of HIV-infected, therapy-naïve men was associated with reduced HIV-specific T cell responses, when both IFN- γ production and proliferation were considered as functional outputs. The relationship of these changes to regulatory T cell populations is likely to be complex, and analysis of this relationship may be confounded by the impact of other chronic viral coinfections. It is unclear whether reduced or impaired HIV-specific T cell immunity contributes to the elevated HIV RNA viral load described in individuals coinfecting with HIV and HSV-2. It will be important to confirm these findings in additional studies and perhaps to examine the effect of HSV-2 therapy on systemic inflammation and HIV-specific immunity.

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