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Human Immunodeficiency Virus Persistence and T-Cell Activation in Blood, Rectal, and Lymph Node Tissue in Human Immunodeficiency Virus–Infected Individuals Receiving Suppressive Antiretroviral Therapy

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Background. Immune activation and inflammation remain elevated in human immunodeficiency virus (HIV)–infected individuals receiving antiretroviral therapy (ART) and may contribute to HIV persistence.

Methods. Using flow cytometry expression of CD38, HLA-DR and PD-1 were measured in blood (n = 48), lymph node (LN; n = 9), and rectal tissue (n = 17) from virally suppressed individuals. Total and integrated HIV DNA, 2-LTR circles, and cell-associated unspliced HIV RNA were quantified.

Results. $CD4^+$ T cells from rectal tissue had a higher frequency of integrated HIV DNA compared with blood (4.26 fold-change in DNA; 95% confidence interval [CI] = 2.61–7.00; $P < .001$) and LN (2.32 fold-change in DNA; 95% CI = 1.22–4.41; $P = .01$). In rectal tissue, there were positive associations between integrated HIV DNA with PD-1^+ $CD4^+$ T-cells (1.44 fold-change in integrated HIV DNA per 10-unit increase in PD-1^+ $CD4^+$ T cells; 95% CI = 1.01–2.05; $P = .045$) and $CD38^+$HLA-DR^+ $CD8^+$ T cells (1.40 fold-change in integrated HIV DNA per 1-unit increase in $CD38^+$HLA-DR^+ $CD8^+$ T cells; 95% CI = 1.05–1.86; $P = .02$). Both associations were independent of current and nadir $CD4^+$ T-cell counts.

Conclusions. During ART, rectal tissue is an important reservoir for HIV persistence with a high frequency of activated $CD4^+$ and $CD8^+$ T cells. PD-1 may represent a marker of HIV persistence in rectal tissue.

Keywords. HIV; HIV persistence; Antiretroviral therapy; T-cell activation; lymph node; rectum; reservoir; PD-1.

Despite the success of combination antiretroviral therapy (ART) in reducing morbidity and mortality, immune activation and inflammation remain elevated in human immunodeficiency virus (HIV)–infected individuals receiving ART compared with HIV-negative individuals [1–3]. Ongoing T-cell activation may play a role in HIV persistence during ART by enhancing low-level residual virus replication through an increased number of activated $CD4^+$ T-cell targets, increasing HIV transcription from an integrated provirus, and/or increasing $CD4^+$ T-cell proliferation [4–6]. Additionally, persistent HIV could be a key driver of ongoing T-cell activation through multiple mechanisms, including direct activation of the innate immune system by HIV RNA as a ligand for Toll-like receptor 7 [5, 7] or persistent presentation of viral antigens to HIV-specific T cells.

Previous studies in HIV-infected individuals on ART have largely described an inverse correlation between $CD4^+$ T-cell count and HIV persistence within the blood, as measured by HIV DNA [4, 8–10]. Expression of the T-cell activation markers HLA-DR and CD38 and immune checkpoint markers, PD-1, TIGIT, TIM-3, and others has been previously associated with HIV persistence on ART, including positive associations with total and integrated HIV DNA [4, 8, 11–13] and cell-associated unspliced (CA-US) HIV RNA [8]. However, multiple other studies have found evidence against a substantial association between markers of T-cell activation and virus persistence [9, 14–17].

Studies in HIV-infected individuals on ART that assessed the relationship of virus persistence and immune activation in gastrointestinal (GI) tract tissue have shown mixed results, similar to blood. Some studies have shown a positive correlation between HIV DNA within mononuclear cells from the recto-sigmoid colon and the percentage of activated $CD8^+$ T cells from this site [9, 16]. Another small study (n = 8) reported the

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opposite findings—with HIV DNA within CD4+ T cells isolated from different sites of the GI tract being negatively associated with T-cell activation markers [11]. To date, there have been no studies examining the relationship between T-cell phenotype and HIV persistence in the lymph node (LN). This tissue is enriched for T follicular helper cells (Tfh), which are highly permissive to HIV infection [18] and may represent a preferential reservoir for HIV during ART [19].

The aim of this study was to determine the relationship between cellular markers of T-cell activation and function to HIV persistence in blood, rectal tissue, and LN in individuals on long-term effective ART. Given the complex effect that CD4+ T-cell lymphopenia could have on both T-cell phenotype and the biology of HIV persistence [20], we chose to study only those individuals on ART with CD4+ T-cell counts >350 cells/μL and also adjusted for current and nadir CD4+ T-cell counts in all analyses.

METHODS

Subject Recruitment

This was a cross-sectional study of 48 HIV-infected adults receiving suppressive ART who were recruited at the University of California San Francisco. The inclusion criteria for the study were receiving ART for >3 years, CD4+ T-cell count >350 cells/μL, and HIV RNA <40 copies/mL for at least 3 years [21]. A subset of these individuals had sigmoidoscopy for rectal tissue (n = 17) and dissection for inguinal LN (n = 9). The study was approved by the institutional research boards at University of California San Francisco, Monash University, and the Alfred Hospital, Melbourne, Australia. All participants provided informed consent.

Flow Cytometry

The following markers were quantified on CD4+ and CD8+ T cells from stored peripheral blood mononuclear cells (PBMCs) using fluorescently conjugated antibodies CD38-APC, HLA-DR-PerCP, or PD-1-APC, together with CD3-Alexa700, CD4-QDot605, and CD8-PB; and from rectal and LN tissue [22] using CD8-QDOT 605, CD4-PE-Texas Red, CD3-V450, PD-1 Alexa Fluor 647, CD38-PE, and HLA-DR-FITC antibodies. Data were acquired on an LSR II Flow cytometer and analyzed using Flow Jo version 9.

Isolation of CD4+ T Cells and Quantification of Human Immunodeficiency Virus Persistence

CD4+ T cells were isolated from stored PBMCs using magnetic depletion per the manufacturer's protocol. CD4+ T cells were isolated from rectal and LN tissue, as described previously [23, 24]. Quantification of HIV DNA (total, integrated, and 2-LTR circles) and CA-US RNA were performed by real-time nested polymerase chain reaction (PCR), as described previously [25, 26].

Statistical Analysis

Data distributions were assessed through descriptive statistics. The level of T-cell activation and PD-1 expression and the frequency of integrated HIV DNA and CA-US HIV RNA were compared between anatomical sites using paired Wilcoxon signed-rank tests and negative binomial regression models [21], respectively. Negative binomial regressions were used to model the effects of the percentage of CD4+ or CD8+ T cells expressing CD38 and HLA-DR or PD-1 on HIV persistence [21]. Models adjusting for the effects of current or nadir CD4+ T-cell counts were included. Within the blood, robust standard errors were used, but not for LN or rectal tissue because of smaller sample sizes [27]. The percentage of CD38+HLA-DR+ CD4+ T cells had evidence of nonlinear effects for models of HIV persistence measures in blood and was therefore log transformed in those models. The percentage of CD38+HLA-DR+ CD4+ and CD8+ T cells varied less in LN and had larger effects than in rectal tissue, so we scaled the presented effects differently to prevent uninterpretable wide or narrow confidence intervals. Models of RNA/DNA ratio also included 2 observations per person, 1 for RNA and 1 for DNA, with a random person effect and an additional predictor being an indicator of whether each observation was for RNA (vs DNA). The effect of T-cell activation on RNA/DNA ratio was obtained as the interaction of the RNA indicator with the percentage of CD4+ or CD8+ T cells expressing CD38 and HLA-DR or PD-1. Analyses were run in Stata version 13.

RESULTS

High Frequency of Activated T Cells in Rectal Tissue Compared With Blood or Lymph Node

Forty-eight participants were recruited for this study, and a subset had rectal (n = 17) and LN (n = 9) tissue collected. Their clinical characteristics are summarized in Table 1. In brief, all individuals had undetectable viral load (<40 copies/mL) and high CD4+ T-cell counts (median = 684; interquartile range [IQR] = 533–858 cells/μL). The percentage of CD4+ and CD8+ T cells that coexpressed T-cell activation markers CD38 and HLA-DR or the immune checkpoint marker PD-1 were measured in all tissues sampled using flow cytometry (Figure 1). Compared with blood, rectal tissue had a higher frequency of CD38+HLA-DR+ CD4+ and CD8+ T cells ($P = .001$ and $P < .001$, respectively) and PD-1+ CD4+ and CD8+ T cells (both $P < .001$). Compared with LN, rectal tissue had a higher frequency of CD38+HLA-DR+ CD8+ T cells and PD-1+ CD4+ T cells (both $P = .04$). The percentage of CD3+HLA-DR+ CD4+ T cells was also higher within the LN than the blood ($P = .008$).

Relationship Between Markers of T-Cell Activation With Current and Nadir CD4+ T-Cell Count

Like previous studies within the blood, we observed statistically significant inverse correlations between current and nadir CD4+ T-cell counts with activated CD4+ T cells (summarized in

Table 1. Clinical Demographics for the Cohort

Parameter	Blood (n = 48)	Rectal tissue (n = 17)	LN (n = 9)
Gender, no. (%)			
Male	46 (96%)	17 (100%)	9 (100%)
Female	1 (2%)	0 (0%)	0 (0%)
Transgender	1 (2%)	0 (0%)	0 (0%)
Age, years	57 (51–62)	58 (50–63)	56 (50–61)
Nadir CD4$^+$ T-cell count, cells/μL	216 (133–385)	147 (92–394)	138 (86–360)
Current CD4$^+$ T-cell count, cells/μL	684 (533–858)	672 (547–801)	521 (412–711)
Current CD4$^+$ T-cell, %	32 (26–41)	30 (24–38)	25 (21–29)
Current CD8$^+$ T-cell count, cells/μL	914 (639–1091)	976 (696–1138)	999 (883–1315)
ART, years	8.5 (5.0–12.4)	11.2 (5.6–13.3)	10.4 (5.3–13.0)

Unless specifically stated, values are listed as median (interquartile range).

Abbreviations: ART, antiretroviral therapy; LN, lymph node.

Supplementary Table 1). There were no substantial relationships between current CD8$^+$ T-cell count and T-cell activation (data not shown). Given these findings, further analyses of the association between HIV persistence and T-cell activation included models that adjusted for current or nadir CD4$^+$ T-cell count.

Human Immunodeficiency Virus Persistence Within CD4$^+$ T Cells Isolated From the Blood and Tissue During Suppressive Antiretroviral Therapy

Human immunodeficiency virus persistence was quantified in total CD4$^+$ T cells isolated from blood, rectal, and LN tissue biopsies. Integrated HIV DNA and CA-US HIV RNA were measured in all sites (Figure 2); total HIV DNA and 2-LTR circles were only quantified in blood. Integrated HIV DNA was highest in the rectal tissue compared with blood (4.26 fold-change; 95% confidence interval [CI] = 2.61–7.00; $P < .001$; n = 19) and with LN (2.32 fold-change; 95% CI = 1.22–4.41; $P = .01$; n = 6). The levels of CA-US HIV RNA were higher in LN (3.25 fold-change; 95% CI = 1.63– 6.50; $P < .001$; n = 6) and rectal (4.45 fold-change; 95% CI = 2.76–10.80; $P < .001$; n = 14) tissue compared with blood.

Relationship Between Human Immunodeficiency Virus Persistence and T-Cell Activation Within Tissue

Next, the relationship between T-cell activation and HIV persistence in the tissue was assessed using negative binomial regression models adjusting for the effects of current or nadir CD4$^+$ T-cell counts (summarized in Tables 2 and 3). In rectal tissue only, there was a strong correlation between integrated HIV DNA and PD-1+ CD4+ T-cells (1.44 fold-change in HIV DNA per 10-unit increase in PD-1+ CD4+ T cells; 95% CI = 1.01–2.05; P = .045) as well as CD38+HLA-DR+ CD8+ T cells (1.40 fold-change in HIV DNA per 10-unit increase in CD38+HLA-DR+ CD8+ T cells; 95% CI = 1.05–1.86; P = .02). Both of these associations were independent of current and nadir CD4$^+$ T-cell counts. There was a positive association between PD-1$^+$ CD4$^+$ T cells and CA-US HIV RNA, which increased from 1.82-fold (95% CI = 1.01–3.28; $P = .047$) to 1.99-fold (95% CI = 1.09–3.65) higher CA-US HIV RNA per 10-unit increase in PD-1$^+$ CD4$^+$ T cells after controlling for the effect of nadir CD4 count ($P = .03$). A marginal positive association between CD38$^+$HLA-DR$^+$ CD8$^+$ T cells and CA-US HIV RNA (1.71 fold-change in CA-US HIV RNA per 10-unit increase in CD38$^+$HLA-DR$^+$ CD8$^+$ T cells; 95% CI = .99–2.97; $P = .06$) was observed, which was independent of both current and nadir CD4$^+$ T-cell counts.

Within the LN, there were positive associations between CD38$^+$HLA-DR$^+$ CD8$^+$ T cells with integrated HIV DNA (1.14 fold-change in HIV DNA; 95% CI = 1.07–1.21) and CA-US HIV RNA (1.22 fold-change in CA-US HIV RNA, 95% CI = 1.15–1.29) per 1-unit increase in CD38$^+$HLA-DR$^+$ CD8$^+$ T cells (both $P < .001$) and independent of current and nadir CD4$^+$ T-cell counts. After controlling for nadir CD4$^+$ T-cell count, there were substantial positive associations between PD-1$^+$ CD8$^+$ T cells with both integrated HIV DNA (5.30 fold-change in HIV DNA; 95% CI = 2.47–10.92) and CA-US HIV RNA (10.35 fold-change in CA-US HIV RNA; 95% CI = 1.83–58.50) per 10-unit increase in PD-1$^+$ CD8$^+$ T cells ($P < .001$ and $P = .008$, respectively). The ratio of CA-US HIV RNA to integrated HIV DNA (CA-US HIV RNA/DNA), which represents the average level of transcription per infected cell [28], was also examined, but no substantial associations were observed (Supplementary Table 2).

Overall, in both sites, there was a strong association of the frequency of CD38$^+$HLA-DR$^+$ CD8$^+$ T cells with HIV integrated DNA and CA-US HIV RNA. In rectal tissue only, there was a strong correlation between PD-1$^+$ CD4$^+$ cells these measures of persistence. All of these associations were independent of current and nadir CD4$^+$ T-cell counts and were surprisingly not observed in LN.

Relationship Between Human Immunodeficiency Virus Persistence and CD4$^+$ T-Cell Activation in Blood

The relationship between HIV persistence and T-cell activation in the blood (n = 48) was next assessed (Table 4). We found that PD-1$^+$ CD4$^+$ T cells were positively associated with total HIV DNA (1.27 fold-change in HIV DNA per

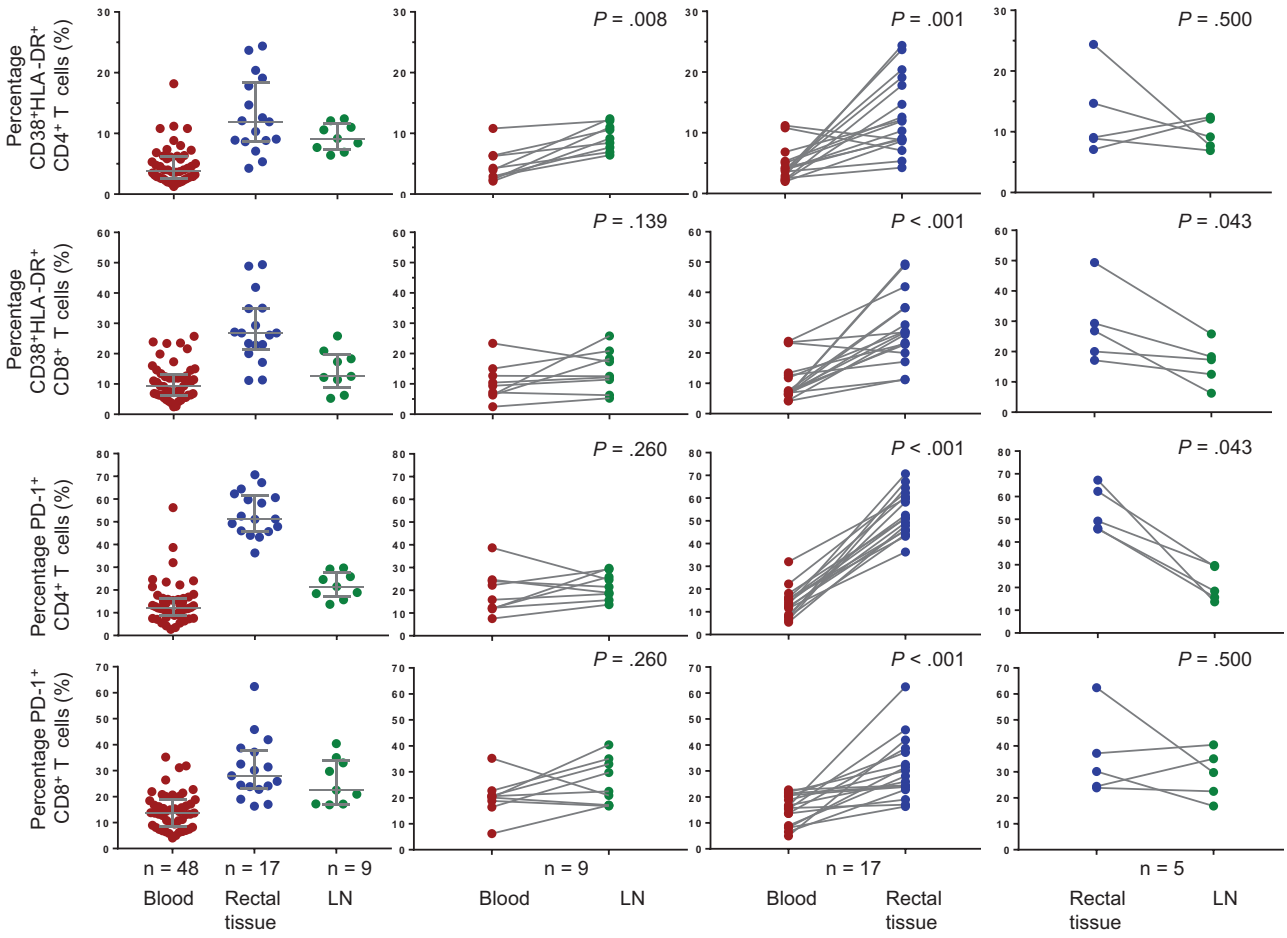

Figure 1. The expression of T-cell immune activation markers within the blood (red), rectal tissue (blue), and lymph node (LN; green) in individuals receiving suppressive antiretroviral therapy (ART). The percentage of CD38$^+$HLA-DR$^+$ CD4$^+$ T cells (first row); CD38$^+$HLA-DR$^+$ CD8$^+$ T cells (second row); PD-1$^+$ CD4$^+$ T cells (third row); and PD-1$^+$ CD8$^+$ T cells (fourth row) is shown. Each symbol represents a different donor. In the left column, all samples are presented from each site—blood (n = 48), rectal tissue (n = 17), lymph node (n = 9)—and the line represents the median and interquartile range. In the other 3 columns, paired analysis between samples isolated from the same individual are presented. The number of pairs is labelled under the *x*-axis. Paired Wilcoxon signed-rank tests were performed.

10-unit increase in PD-1$^+$ CD4$^+$ T cells; 95% CI = 1.08–1.50; $P = .003$), and this was independent of both current and nadir CD4$^+$ T-cell counts. There was a moderate negative association between CA-US HIV RNA and CD38$^+$HLA-DR$^+$ CD4$^+$ T-cells (0.74 fold-change in CA-US HIV RNA per 2-fold increase in the percentage of CD38$^+$HLA-DR$^+$ CD4$^+$ T cells; (95% CI = .53–1.02; $P = .07$), which was strengthened after adjustment for current or nadir CD4$^+$ T-cell counts (0.67 fold-change in CA-US HIV RNA, 95% CI = .48–.92, $P = .01$; and 0.73 fold-change in CA-US HIV RNA, 95% CI = .54–.99, $P = .04$), respectively.

Coexpression of CD38 and HLA-DR was inversely associated with 2-LTR circles (0.61 fold-change in 2-LTR circles; 95% CI = .36–1.02), which was strengthened after adjusting for nadir CD4$^+$ T-cell count (0.58 fold-change in 2-LTR circles; 95% CI = .31–.96; $P = .01$). Expression of CD38 and HLA-DR or PD-1 was positively associated with integrated HIV DNA, but after adjusting for current CD4$^+$ T-cell count, these associations were no longer statistically significant.

The ratio of CA-US HIV RNA/DNA was also examined (Supplementary Table 2). We observed inverse associations between the CA-US HIV RNA/DNA ratio and the proportion of CD38$^+$HLA-DR$^+$ CD4$^+$ T cells (0.84 fold-change HIV RNA/DNA per 1-unit increase of CD38$^+$HLA-DR$^+$ CD4$^+$ T cells; 95% CI = .76–.92; $P < .001$) and the proportion of PD-1$^+$ CD4$^+$ T cells (0.87 fold-change HIV RNA/DNA per 1-unit increase of PD-1$^+$ CD4$^+$ T cells; 95% CI = .77–.99; $P = .03$).

Taken together, there was a clear association between CD4$^+$ T-cell activation and virus persistence in blood, with an inverse relationship between activated CD4$^+$ T cells and the HIV RNA/DNA ratio and 2-LTR circles.

Relationship Between Human Immunodeficiency Virus Persistence and CD8$^+$ T-Cell Activation in Blood

For CD8$^+$ T-cells (Table 5), there was a marginal statistically significant positive association between CD38$^+$HLA-DR$^+$ CD8$^+$ T-cells and total HIV DNA (1.38 fold-change in HIV DNA per 10-unit increase in CD38$^+$HLA-DR$^+$ CD8$^+$ T cells; 95% CI = 1.00–1.19;

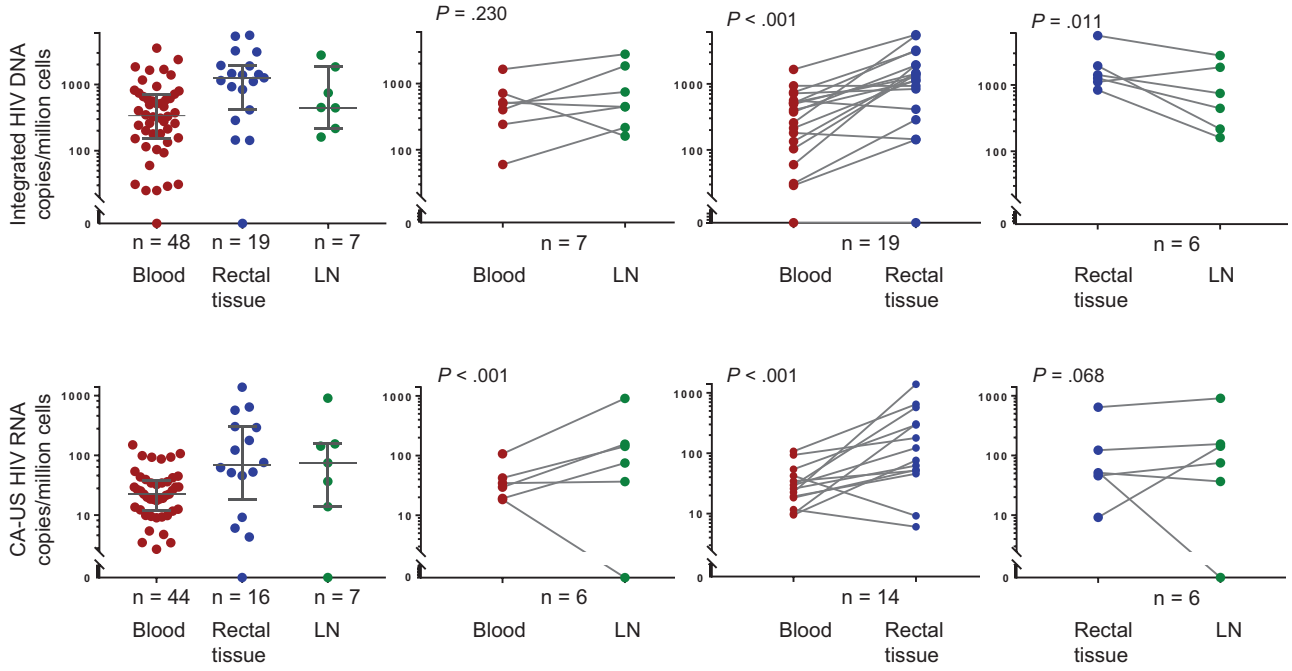

Figure 2. Integrated human immunodeficiency virus (HIV) DNA and CA-US HIV RNA were quantified in CD4+ T cells isolated from the blood (red), rectal tissue (blue), and lymph node (LN; green) in individuals receiving suppressive antiretroviral therapy (ART). Each symbol represents a different donor. The left columns show all samples from each site for integrated HIV DNA (top row) and CA-US HIV RNA (bottom row). The line represents the median and interquartile range. In the other 3 columns, paired comparisons of the different tissue sites are shown. The number of pairs is labelled under the *x*-axis. Mixed effects negative binomial regression analyses are shown as previously described [21].

P = .051), which was independent of current and nadir CD4+ T-cell counts. An inverse association was observed between the CA-US HIV RNA/DNA ratio and the proportion of CD38+HLA-DR+ CD8+ T-cells (0.92 fold-change HIV RNA/DNA per 1-unit increase of CD38+HLA-DR+ CD8+ T cells; 95% CI = .87–.98; *P* = .005) (Supplementary Table 2). There were no other substantial associations between HIV persistence and CD8+ T-cell activation.

DISCUSSION

Several studies have demonstrated relationships between markers of T-cell activation and HIV persistence in blood in HIV-infected individuals on ART, which we confirmed in this study. We extend these previous studies by examining blood, rectal, and LN tissue in a cohort of individuals with robust immune reconstitution.

Table 2. Negative Binomial Regression Models Assessing the Relationships Between Human Immunodeficiency Virus Persistence and T-Cell Activation Within Rectal Tissue

		Unadjusted		Adjusted for current CD4+		Adjusted for nadir CD4+	
Outcome	Predictor (percentage of[a])	Fold effect (95% CI)	*P* value	Fold effect (95% CI)	*P* value	Fold effect (95% CI)	*P* value
Integrated HIV DNA[b]	CD38+HLA-DR+CD4+ T cells	1.48 (.74–2.97)	.27	1.65 (.70–3.88)	.25	1.51 (.74–3.08)	.25
	PD1+ CD4+ T cells	1.44 (1.01–2.05)	**.045**	1.43 (1.01–2.04)	**.046**	1.44 (1.01–2.05)	**.04**
	CD38+HLA-DR+ CD8+ T cells	1.40 (1.05–1.86)	**.02**	1.42 (1.04–1.94)	**.03**	1.46 (1.10–1.93)	**.008**
	PD1+ CD8+ T cells	1.23 (.92–1.64)	.17	1.24 (.93–1.64)	.14	1.24 (.93–1.65)	.15
CA-US HIV RNA[c]	CD38+HLA-DR+CD4+ T cells	2.35 (.59–9.32)	.23	2.55 (.63–1.37)	.19	2.17 (.52–9.05)	.29
	PD1+ CD4+ T cells	1.82 (1.01–3.28)	**.047**	1.84 (1.03–3.29)	**.04**	1.99 (1.09–3.65)	**.03**
	CD38+HLA-DR+ CD8+ T cells	1.71 (.99–2.97)	.06	1.73 (.99–3.02)	.054	1.68 (.95–2.97)	.07
	PD1+ CD8+ T cells	1.46 (.77–2.77)	.24	1.57 (.79–3.10)	.20	1.64 (.83–3.27)	.16

Result interpretation: Fold-change in the outcome (marker of HIV persistence) per 10-unit difference in the predictor (marker of T-cell activation). Statistically significant *P* values < .05 are in bold.

Abbreviation: CI, confidence interval.

[a]Percentage CD4+ or CD8+ T cells that express activation markers.

[b] Integrated HIV DNA units copies/million CD4+.

[c]CA-US HIV RNA units HIV RNA copies/million 18s copies.

Table 3. Negative Binomial Regression Models of the Relationships Between Human Immunodeficiency Virus Persistence and T-Cell Activation Within the Lymph Node

Outcome	Predictor (percentage of[a])	Unadjusted		Adjusted for Current CD4+		Adjusted for Nadir CD4+	
		Fold effect (95% CI)	*P* value	Fold effect (95% CI)	*P* value	Fold effect (95% CI)	*P* value
Integrated HIV DNA[b]	CD38+HLA-DR+ CD4+ T cells*	1.06 (.75–1.50)	.74	0.99 (.74–1.33)	.95	1.19 (.79–1.79)	.41
	PD1+ CD4+ T cells#	0.72 (.28–1.84)	.49	0.81 (.32–2.08)	.67	0.73 (.22–2.44)	.60
	CD38+HLA-DR+ CD8+ T cells*	1.14 (1.07–1.21)	**<.001**	1.20 (1.12–1.28)	**<.001**	1.15 (1.08–1.21)	**<.001**
	PD1+ CD8+ T cells#	2.16 (.90–5.16)	.08	1.56 (.42–5.79)	.50	5.30 (2.57–1.92)	**<.001**
CA-US HIV RNA[c]	CD38+HLA-DR+ CD4+ T cells*	0.97 (.45–2.08)	.94	0.76 (.42–1.36)	.36	1.49 (.45–4.93)	.51
	PD1+ CD4+ T cells#	0.36 (.09–1.03)	.13	0.33 (.09–1.17)	.09	0.21 (.04–1. 26)	.09
	CD38+ HLA-DR+ CD8+ T cells*	1.22 (1.15–1.29)	**<.001**	1.27 (1.17–1.38)	**<.001**	1.23 (1.17–1.30)	**<.001**
	PD1+ CD8+ T cells	2.25 (.49–1.34)	.30	0.32 (.04–2.29)	.26	1.35 (1.83–58.50)	**.008**

Result interpretation: Fold-change in the outcome (marker of HIV persistence) per *1-unit or #10-unit difference in the predictor (marker of T-cell activation). Statistically significant *P* values < .05 are bold.

Abbreviation: CI = confidence interval.

[a]Percentage CD4+ or CD8+ T cells that express activation markers.

[b]Integrated HIV DNA units copies/million CD4+.

[c]CA-US HIV RNA units HIV RNA copies/million 18s copies.

Using negative binomial regression models adjusting for current or nadir CD4+ T-cell count, statistically significant positive associations were observed in rectal tissue and LN between the percentage of CD38+HLA-DR+ CD8+ T cells and both integrated HIV DNA and CA-US HIV RNA, and effects were larger within the LN (note that these effects in Table 3 are scaled per 1 unit, vs per 10 units in Table 2). We also observed statistically significant positive associations between the frequency of PD-1+ CD4+ T cells in blood with total HIV DNA and in rectal tissue with integrated HIV DNA and CA-US HIV RNA, but these associations were not statistically significant in the LN.

During chronic HIV infection, PD-1 expression is upregulated on T cells and is associated with markers of T-cell activation, proliferation, and exhaustion [29–31]. PD-1 is an immune checkpoint marker that represses immune responses by blocking T-cell activation, proliferation, and effector function by inducing a resting state [32], making these cells potential targets for HIV latency and persistence [4, 13, 32]. In support of this mechanism, we previously demonstrated in an in vitro model of myeloid dendritic cell induced latency that latently infected CD4+ T-cells expressed high levels of PD-1 [33]. In addition, in HIV-infected individuals receiving ART, central

Table 4. Negative Binomial Regression Models to Assess the Relationship Between Human Immunodeficiency Virus Persistence and CD4+ T-Cell Activation Within the Blood

Outcome	Predictor (percentage of[a])	Unadjusted		Adjusted for Current CD4+		Adjusted for Nadir CD4+	
		Fold effect (95% CI)	*P* value	Fold effect (95% CI)	*P* value	Fold effect (95% CI)	*P* value
Integrated HIV DNA[b]	CD38+HLA-DR+ CD4+ T cells*	1.31 (1.01–1.71)	**.04**	1.00 (.74–1.35)	.99	1.37 (1.06–1.77)	**.02**
	PD1+ CD4+ T cells	1.34 (1.12–1.59)	**.001**	1.15 (.97–1.35)	.11	1.32 (1.13–1.54)	**.001**
Total HIV DNA[c]	CD38+HLA-DR+ CD4+ T cells*	1.06 (.82–1.37)	.67	0.96 (.72–1.28)	.78	1.09 (.83–1.41)	.54
	PD1+ CD4+ T cells	1.27 (1.08–1.50)	**.003**	1.22 (1.05–1.43)	**.01**	1.26 (1.09–1.46)	**.002**
2-LTR circles[d]	CD38+HLA-DR+ CD4+ T cells*	0.61 (.36–1.02)	.06	0.55 (.31–.96)	**.04**	0.58 (.38–.89)	**.01**
	PD1+ CD4+ T cells	0.98 (.54–1.77)	.94	1.14 (.50–2.59)	.75	0.86 (.66–1.10)	.23
CA-US HIV RNA[e]	CD38+HLA-DR+ CD4+ T cells*	0.74 (.53–1.02)	.07	0.67 (.48–.92)	**.01**	0.73 (.54–.99)	**.04**
	PD1+ CD4+ T cells	1.00 (.70–1.41)	.99	0.97 (.62–1.53)	.90	0.96 (.68–1.37)	.83

Result interpretation: fold-change in the outcome (marker of HIV persistence) per 10-unit difference in the predictor (marker of T-cell activation) except for when the predictor was CD38+ HLA-DR+ CD4+ T cells, which was log2 transformed to better model its effect; results here are interpreted as the change in the outcome per 2-fold increase of the predictor, as noted by *. Statistically significant *P* values < .05 are bold.

Abbreviation: CI, confidence interval.

[a]Percentage CD4+ T cells that express activation markers.

[b]Integrated HIV DNA

[c]total HIV DNA

[d]2-LTR circles units copies/million CD4+.

[e]CA-US HIV RNA copies/million 18s copies.

Table 5. Negative Binomial Regression Models to Assess the Relationships Between Human Immunodeficiency Virus Persistence and CD8⁺ T-Cell Activation Within the Blood

		Unadjusted		Adjusted for Current CD4⁺		Adjusted for Nadir CD4⁺	
Outcome	Predictor (Percentage of)	Fold effect (95% CI)	*P* value	Fold effect (95% CI)	*P* value	Fold effect (95% CI)	*P* value
Integrated HIV DNA[a]	CD38⁺HLA-DR⁺ CD8⁺ T cells	1.36 (.80–2.32)	.25	1.11 (.66–1.86)	.70	1.44 (.87–2.40)	.16
	PD1⁺ CD8⁺ T cells	0.94 (.61–1.46)	.78	0.99 (.61–1.59)	.95	0.88 (.57–1.36)	.57
Total HIV DNA[b]	CD38⁺HLA-DR⁺ CD8⁺ T cells	1.38 (1.00–1.90)	.051	1.30 (.94–1.80)	.11	1.40 (1.02–1.91)	**.04**
	PD1⁺ CD8⁺ T cells	1.20 (.88–1.64)	.26	1.18 (.87–1.60)	.29	1.15 (.83–1.60)	.40
2-LTR circles[c]	CD38⁺HLA-DR⁺ CD8⁺ T cells	1.17 (.50–2.75)	.72	1.30 (.56–3.05)	.54	1.01 (.48–2.15)	.97
	PD1⁺ CD8⁺ T cells	2.10 (1.11–3.97)	**.02**	2.16 (1.16–4.03)	**.02**	1.59 (.77–3.28)	.21
CA-US HIV RNA[d]	CD38⁺HLA-DR⁺ CD8⁺ T cells	0.74 (.51–1.09)	.13	0.70 (.46–1.07)	.10	0.73 (.51–1.03)	.08
	PD1⁺ CD8⁺ T cells	1.24 (.83–1.84)	.29	1.23 (.81–1.88)	.33	1.21 (.80–1.81)	.37

Result interpretation: Fold-change in the outcome (marker of HIV persistence) per 10-unit difference in the predictor (marker of T-cell activation). Percentage CD8⁺ T cells that express activation markers. Statistically significant *P* values < .05 are bold.

Abbreviation: CI, confidence interval.

[a]Integrated HIV DNA

[b]total HIV DNA

[c]2-LTR circles units copies/million CD4⁺.

[d]CA-US HIV RNA units HIV RNA copies/million 18s copies.

and transitional memory CD4⁺ T cells that express high levels of PD-1 preferentially harbor HIV DNA [4, 13]. Aligned with these findings, we observed positive associations in PD-1⁺ CD4⁺ T cells from rectal tissue and integrated HIV DNA. These associations were only seen in CD4⁺ T cells from rectal tissue and not LN. In contrast, within the blood there was an inverse association between the frequency of PD-1⁺ CD4⁺ T cells and the CA-US RNA/DNA ratio, potentially consistent with a role for PD-1 in the suppression of viral transcription.

We did not observe a positive association between PD-1⁺ CD4⁺ T cells and HIV persistence within the LN, where PD-1 is expressed at high levels on Tfh, which predominantly reside within the germinal centers. Given that we used whole LN for cell extraction and not LN aspirates, we think it is likely that our tissue sampling included B-cell follicles, which are enriched for infected Tfh. There are several potential explanations for our findings. First, if productive infection of Tfh persists on ART, then it is possible that these cells die and are subsequently depleted. Recent work suggests that HIV-infected Tfh decay with prolonged ART [19], and the patients in this study were on ART for 8–10 years. Second, Tfh are only a subset of PD-1hi cells (also expressing CXCR5), and here we only measured PD-1 and not CXCR5 [18, 34]. Finally, we examined only a small cohort of individuals for LN tissue; however, we found strong associations between markers of HIV persistence and other activation markers, including CD38 and HLA-DR, but not with PD-1 expression.

Studies of both HIV and simian immunodeficiency virus (SIV) infection have demonstrated that infection of Tfh within the LN is established early in infection [18, 35]. Tfh are also found at high frequency in the Peyer's patches of the rectal mucosa and have been identified as preferential targets of SIV infection [36]. These cells have the capacity for long-term survival through elevated expression of Bcl-2 and increased proliferation [36]. Our study demonstrated that the highest proportion of PD-1⁺ CD4⁺ T cells was found within the rectal tissue. Although most studies in HIV-infected individuals have focused on isolating Tfh from the LN, other tissue sites, including the rectal mucosa, ileum, and spleen, may also be important locations for Tfh [18, 35–38]. Further phenotypic analysis of cells isolated from the rectal mucosa needs to be performed to confirm that the high proportion of PD-1⁺ CD4⁺ T cells detected are indeed Tfh and that these cells reside within other tissue sites in humans. In addition, Tfh precursor cells, which also express PD-1, can be found circulating within the blood and could also be preferential targets for HIV infection and persistence [34]. Our findings suggests that PD-1 may represent an important marker of HIV persistence in rectal tissue during suppressive ART in individuals treated during chronic infection, independent of time of treatment and successful immune recovery.

The relationships between integrated and total DNA and markers of T-cell activation were different, even though most HIV DNA should be integrated in individuals on ART [4, 39]. We believe this was due to the assays measuring different virus intermediates. The Alu-HIV PCR assay used to quantify integrated HIV DNA may not detect some integrated genomes, particularly when the integration site is far from an Alu sequence within the human genome [40]. The total DNA PCR assay will detect linear and circular forms of unintegrated DNA as well as integrated DNA [26]. The relative levels of each of these forms of HIV DNA may differ in individuals. Finally, the associations described here are likely to reflect T-cell proliferation, which will lead to

the persistence of integrated HIV DNA following cell division, whereas unintegrated DNA will become progressively diluted.

Like previous studies, we demonstrated a higher proportion of activated T cells within tissues, specifically the LN and the rectal tissue [11] compared with the blood. Increased frequency of activated T cells in rectal tissue compared with blood has also been observed in HIV-uninfected individuals [41–43]. Our study was the first to assess the relationship of markers of T-cell activation with markers of HIV persistence within the LN and rectal tissue from the same individual. We found statistically significant positive associations between activated $CD38^+HLA\text{-}DR^+$ $CD8^+$ T cells and the concentration of CA-US HIV RNA and integrated HIV DNA within $CD4^+$ T-cells isolated from both LN and rectal tissue. Why would there be a positive relationship between markers of $CD8^+$ T-cell activation and virus persistence? We propose several explanations for this finding [20, 44]. The presence of activated $CD8^+$ T cells within the tissue in this setting could potentially be driven by ongoing replication and HIV antigen presentation. Given that penetration of antiretroviral drugs to the LN and rectal tissue is reduced [45] and there are immune-privileged sites in LN such as B-cell follicles with limited migration of HIV-specific cytotoxic T cells [46], these data suggest that the presence of activated $CD8^+$ T cells could reflect ongoing viral expression or replication or release of virus from HIV-infected cells in the LN and rectal tissue during ART. Alternatively, chronic inflammation may result in upregulation of counter-regulatory immunosuppressive responses, including upregulation of PD-1 and/or activation of T regulatory cells. This would result in reduced capacity of $CD8^+$ T cells to control or clear the reservoir. Defining why $CD8^+$ T-cell activation correlates with HIV persistence in tissues could lead to development of novel therapeutic interventions.

There are some limitations to our study. First, we quantified HIV persistence within total $CD4^+$ T cells, which included a mix of resting and activated $CD4^+$ T-cells, but there is increasing evidence that HIV persists in multiple T-cell subsets on ART, including activated T cells [47]. Second, given this study was a cross-sectional observational study, we were unable to determine whether immune activation drives HIV persistence or vice versa, and it is likely these pathways are interrelated [20, 44, 48]. Finally, we only used PCR-based measures for HIV persistence. Given the recent reports of high frequency of defective viral genomes on ART [49], it is important to also determine whether persistent HIV on ART is inducible [50] or replication competent [49], but this was not possible in this study because of limited cell numbers. It is possible that the replication-competent reservoir may vary in size in different tissue sites, but we were unable to assess this here.

In conclusion, different markers of T-cell activation correlated with virus persistence in blood and tissue, and many associations were independent of current or nadir $CD4^+$ T-cell count. Rectal tissue is an important site for HIV persistence and T-cell activation in HIV-infected individuals on ART. The strong association between the frequency of $PD\text{-}1^+CD4^+$ T cells and virus persistence suggests that PD-1 may represent an important marker of HIV persistence at this site.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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