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MAJOR ARTICLE

Plasma Indoleamine 2,3-Dioxygenase Activity Is Associated With the Size of the Human Immunodeficiency Virus Reservoir in Patients Receiving Antiretroviral Therapy

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Background. Indoleamine 2,3-dioxygenase (IDO) is an immunoregulatory enzyme that metabolizes tryptophan to immunosuppressive kynurenines. We investigated whether IDO activity is associated with the size of HIV reservoir.

Methods. Total human immunodeficiency virus (HIV) DNA in peripheral blood mononuclear cells (PBMCs) from 127 HIV-infected patients receiving antiretroviral therapy (ART) was quantified. Tryptophan and kynurenine concentrations, as well as microbial translocation markers, were measured in plasma samples. T-cell activation and exhaustion in PBMCs were assessed by flow cytometry.

Results. Elevated IDO activity prior to ART correlated with on-ART HIV DNA ($r = 0.35$, $P = .004$), but was not associated with pre-ART HIV DNA. A median duration of 15 months of ART significantly decreased IDO activity; however, these levels were still higher than those observed in HIV-uninfected controls. Among treated participants, IDO activity positively correlated with their concurrent HIV DNA ($r = 0.36$, $P < .0001$). Multivariate model showed an independent association of pre-ART CD4/CD8 ratio (adjusted odds ratio [aOR], 0.75 per 0.1 increase [95% confidence interval {CI}, .62–.91]) and on-ART IDO activity (aOR, 1.09 per nM/μM increase [95% CI, 1.04–1.14]) with higher levels of HIV DNA on-ART. A lack of association of the microbial translocation markers was observed with the size of HIV reservoir. HIV DNA positively correlated with the proportions of activated CD4 T and CD8 T cells and exhausted CD4 T cells.

Conclusions. We observed a positive correlation between IDO activity and total HIV DNA in blood, highlighting the important role of immunometabolic aberrations in HIV persistence.

Keywords. HIV reservoir; indoleamine 2,3-dioxygenase; kynurenine; microbial translocation; ART.

Human immunodeficiency virus (HIV) reservoirs in resting CD4 T cells represent the major obstacle to an HIV cure owing to their persistence on effective antiretroviral therapy (ART) [1–3]. The reservoir is established early in HIV infection, which allows it to undergo rapid viral replication upon ART interruption. Such viral rebound has also been witnessed in patients initiating ART at a very early stage [4, 5]. Furthermore, Siliciano et al have calculated that the reservoir is stable with an estimated half-life of 44 months and will require >70 years of ART to eradicate [6]. Following ART discontinuation, HIV rebounds within 4 weeks in majority of patients and such time to rebound can be predicted by the size of the HIV reservoir on ART [7, 8]. To develop a cure, identifying factors that influence the size of HIV reservoir remains a research priority [9].

We and others have reported that indoleamine 2,3-dioxygenase (IDO) activity is elevated during acute HIV infection and that such elevation persists during the chronic phase and is not normalized in patients receiving long-term suppressive ART [10–12]. IDO is a rate-limiting enzyme in the kynurenine pathway that is induced by interferon-γ. IDO metabolizes tryptophan to immunosuppressive kynurenines, and thereby is implicated in the pathogenesis of cancers and infectious diseases [13]. During HIV infection, elevated expression of IDO skews CD4 T-cell differentiation into regulatory T cells instead of T-helper 17 cells and directly impairs T-cell immune responses, thus contributing to HIV disease progression [14–16]. As an immune checkpoint, IDO and its downstream kynurenine pathway are increasingly receiving attention for being immunotherapeutic targets along with programmed cell death 1 (PD-1) blockade. Therefore, IDO activity may play a role in HIV persistence and deserves further investigation.

While most previous studies suggest that chronic inflammation and immune activation contribute to HIV persistence, there

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have also been conflicting reports [17–21]. Chronic inflammation might lead to HIV persistence by generating new target cells and increasing the proliferation of infected cells [22]. In HIV-infected individuals, viral replication and persistently elevated levels of inflammation and T-cell activation may be consequences of gut microbial translocation [23]. The pre-ART elevated IDO activity was also associated with plasma levels of microbial translocation markers [11]. Whether microbial translocation contributes to HIV persistence is not yet established. Several soluble markers of microbial translocation, such as gram-negative bacterial cell wall component lipopolysaccharide (LPS) and the host acute response proteins LPS-binding protein (LBP) and soluble CD14 (sCD14), have been studied mostly in cross-sectional studies that reported divergent results [21]. Therefore, more comprehensive study of markers of immune dysfunction and microbial translocation is warranted to determine their role in HIV persistence.

Herein, we quantified total HIV type 1 (HIV-1) DNA levels in relation to IDO activity, microbial translocation, immune activation, and T-cell exhaustion in a cohort of HIV-infected participants receiving first-line ART.

METHODS

Study Population and Sample Size

The study population consisted of 127 HIV-1–infected patients without any history of opportunistic infections or cancers, who initiated first-line ART for a median duration of 15 (interquartile range [IQR], 12–27) months. The study population did not include any subjects with known autoimmune disease and/or pregnant women. Whole blood samples (10 mL) were collected from all participants to quantify total HIV DNA. Pre-ART plasma samples were available in 64 of the participants, which were quantified together with their on-ART samples to investigate the changes in IDO activity and the markers of microbial translocation. In addition, 25 HIV-uninfected controls were studied as a comparison group for the levels of microbial translocation and IDO activity. Clinical records of these patients were reviewed for the past medical history and ART regimens. Ethics approval was obtained from the Shanghai Public Health Clinical Center Ethic Committee, China. Informed consent was obtained from all study participants.

Quantification of HIV RNA and CD4 and CD8 T-Cell Counts

CD4 and CD8 T-cell counts were assessed by flow cytometry (BD Biosciences, Franklin Lakes, New Jersey) and HIV-1 RNA loads measured by polymerase chain reaction (PCR) (Cobas Amplicor, Roche, Basel, Switzerland) at the clinical laboratory of the Shanghai Public Health Clinical Center.

Quantification of Tryptophan and Kynurenine Levels in Plasma as a Measure of IDO Activity

Plasma levels of tryptophan and kynurenine were quantified using ultra-performance liquid chromatography–mass spectrometry as previously described [24]. IDO activity was calculated as the plasma kynurenine/tryptophan ratio (K/T ratio).

Quantification of Soluble Markers of Microbial Translocation and Immune Activation

Plasma levels of markers of microbial translocation including LPS (CUSABIO Life Science, Wuhan, China), endogenous endotoxin-core antibody (EndoCAb) (Hycult Biotech, the Netherlands), and LBP (Hycult Biotech), sCD14 (R&D Systems, Minneapolis, Minnesota), and soluble CD163 (sCD163) (R&D Systems) were determined using enzyme-linked immunosorbent assays according to the manufacturers' instructions.

Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated and stained with the following antibodies: CD3 APC-H7, CD4 FITC, CD8 APC, CD38 PE-Cy7, HLA-DR PerCP-Cy5.5, PD-1 PE, and 7AAD PE-Cy5 (all from BD Biosciences). Cells were fixed in 1% paraformaldehyde and analyzed within 24 hours of staining. Data were analyzed using FlowJo software version 10 (FlowJo, LLC, Ashland, Oregon).

Determination of HIV DNA

HIV-1 DNA was amplified and quantified using a fluorescence-based, real-time HIV quantitative detection kit (SUPBIO, Guangzhou, China) after extraction of total cellular DNA from peripheral blood using a DNA isolation kit (Qiagen, Valencia, California). The quantification range of this assay was $10–5 \times 10^6$ copies/10^6 PBMCs. Levels of total HIV DNA below the lower limit of detection were deemed as 10 copies/10^6 PBMCs.

Statistical Analyses

The normality of the data was assessed using the Shapiro-Wilk test. Normally distributed data were described as the mean ± standard deviation while nonnormally distributed variables were described as median with IQR. For comparisons between baseline and follow-up samples, paired *t* test or Wilcoxon signed-rank test was performed depending on the distribution of the variable. Spearman rank correlation test was used to measure the association between HIV DNA and other variables. All the associations were corrected for false discovery rate <0.05 using the Benjamini-Hochberg method (reported as *q* value). HIV DNA levels were categorized into lower HIV DNA group and higher HIV DNA group using the median as a cutoff. Binary logistic regression was used to determine the independent association of clinical covariates with HIV DNA. All analyses were performed using Stata version 12.0 (StataCorp, College Station, Texas) and GraphPad Prism 7.0 (GraphPad Software, La Jolla, California) software.

RESULTS

Demographic and Clinical Characteristics of the Study Participants

Among the 127 enrolled HIV-infected patients, 92.1% (117/127) were male, with a median age of 32 (IQR, 27–44) years (Table 1). The majority of the participants received 2 nucleoside reverse transcriptase inhibitors plus 1 nonnucleoside reverse transcriptase inhibitor–based regimen. HIV RNA was below the limit of

Table 1. Study Participants

Characteristics	Total (N = 127)
Age at ART initiation, y, median (IQR)	32 (27–44)
Male sex, No. (%)	117 (92.1)
Pre-ART CD4 T-cell count, cells/μL, median (IQR)	348 (258–459)
Pre-ART CD4/CD8 ratio, median (IQR)	0.36 (0.25–0.49)
Pre-ART HIV RNA[a], log_{10} copies/mL, median (IQR)	4.61 (4.15–5.05)
ART regimen, No. (%)	
TDF + 3TC + EFV	116 (91.3)
ZDV + 3TC + EFV/NVP	7 (5.5)
TDF + 3TC + RAL	2 (1.6)
TDF + 3TC + LPV/r	2 (1.6)
ART duration, mo, median (IQR)	15 (12–27)
On-ART CD4 T-cell count, cells/μL, median (IQR)	403 (332–560)
On-ART CD4/CD8 ratio, median (IQR)	0.62 (0.45–0.87)

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy, EFV, efavirenz; HIV, human immunodeficiency virus; IQR, interquartile range; LPV/r, lopinavir/ritonavir; NVP, nevirapine; RAL, raltegravir; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

[a]Data from 99 participants.

detection (<20 copies/mL) in all the participants after a median of 15 (IQR, 12–27) months of ART. The on-ART total HIV DNA level in this cohort was 2.27 (IQR, 1.92–2.62) log_{10} copies/10^6 PBMCs.

Pre-ART CD4/CD8 Ratio Predicted On-ART HIV DNA Level

The age and sex did not influence the size of the HIV reservoir on-ART (Table 2). Pre-ART HIV RNA was marginally correlated with on-ART HIV DNA ($r = 0.19$, $P = .059$). Pre-ART CD4 T-cell counts and pre-ART CD4/CD8 ratio inversely predicted, while pre-ART CD8 T-cell counts positively predicted, on-ART HIV DNA ($r = –0.27$, $P = .003$; $r = –0.41$, $P < .0001$; and $r = 0.22$, $P = .013$, respectively). On-ART CD4 T-cell counts and CD4/CD8 ratio increased substantially, the latter of which was associated with the duration of ART. As expected, on-ART HIV DNA inversely correlated with the duration of ART ($r = –0.38$, $P < .0001$). In addition, on-ART CD8 T-cell counts and on-ART CD4/CD8 ratio correlated with their concurrent HIV DNA levels ($r = 0.32$, $P = .0003$ and $r = –0.41$, $P < .0001$, respectively). After adjusting for false discovery rate, all of these factors were still significantly associated with on-ART HIV DNA.

IDO Activity Was Correlated With On-ART HIV DNA

IDO activity was significantly elevated in HIV-infected patients before ART as compared to HIV-uninfected controls (Figure 1A). The elevated pre-ART IDO activity positively correlated with pre-ART HIV RNA ($r = 0.46$, $P = .0002$) and positively predicted on-ART HIV DNA level ($r = 0.35$, $P = .004$; Figure 1B and Table 2). To explore the association between pre-ART IDO activity and pre-ART HIV DNA, another 41 patients with plasma and PBMCs collected at the time of ART initiation were studied (Supplementary Table 1). Strikingly, pre-ART IDO activity was not associated with pre-ART HIV DNA in this group ($r = –0.08$, $P = .601$; Figure 1C).

IDO activity decreased significantly on ART. However, IDO activity in HIV-infected participants was still higher than that in HIV-uninfected controls. On-ART IDO activity was not associated with any of the concurrent microbial translocation markers (data not shown). The on-ART IDO activity strongly correlated with on-ART HIV DNA ($r = 0.36$, $P < .0001$; Figure 1D). The correlation was stronger among participants with pre-ART CD4

Table 2. False Discovery Rate Adjusted *P* Value (*q* Value) for Each Association Calculated

Characteristic	*P* Value	*q* Value
Association with on-ART HIV DNA		
Age	.996	.798
Sex	.783	.681
Pre-ART HIV RNA	.059	.112
Pre-ART CD4 T-cell count	.003	**.011**
Pre-ART CD8 T-cell count	.013	**.040**
Pre-ART CD4/CD8	.000002	**.00003**
Duration of ART	.00001	**.0001**
On-ART CD4 T-cell count	.039	.079
On-ART CD8 T-cell count	.0003	**.002**
On-ART CD4/CD8	.000001	**.00003**
Pre-ART IDO activity	.004	**.016**
On-ART IDO activity	.00003	**.0003**
On-ART IDO activity (CD4 T-cell count <200 cells/μL)	.011	**.037**
Pre-ART sCD14	.556	.546
Pre-ART LPS	.537	.546
Pre-ART LBP	.897	.738
Pre-ART EndoCAb	.546	.546
Pre-ART sCD163	.398	.520
On-ART sCD14	.348	.481
On-ART LPS	.461	.520
On-ART LBP	.783	.681
On-ART EndoCAb	.897	.738
On-ART sCD163	.033	.072
Percentage of HLA-DR^+CD38^+ CD4	.028	.065
Percentage of HLA-DR^+CD38^+ CD8	.026	.065
Percentage of PD-1^+ CD4	.026	.065
Percentage of PD-1^+ CD8	.455	.520
Association with pre-ART IDO activity		
Pre-ART HIV DNA	.601	.571
Pre-ART HIV RNA	.0002	**.001**
Association with on-ART IDO activity		
On-ART sCD14	.263	.397
On-ART LPS	.429	.520
On-ART LBP	.457	.520
On-ART EndoCAb	.757	.681
On-ART sCD163	.274	.397
Percentage of HLA-DR^+CD38^+ CD4	.211	.337
Percentage of HLA-DR^+CD38^+ CD8	.519	.546
Percentage of PD-1^+ CD4	.083	.148
Percentage of PD-1^+ CD8	.116	.196

Data in bold indicate significant after adjusted for false discovery rate. False discovery rate <0.05.

Abbreviations: ART, antiretroviral therapy; EndoCAb, endogenous endotoxin-core antibody; HIV, human immunodeficiency virus; HLA-DR, human leukocyte antigen – DR isotype; IDO, indoleamine 2,3-dioxygenase; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; PD-1, programmed cell death 1; sCD14, soluble CD14; sCD163, soluble CD163.

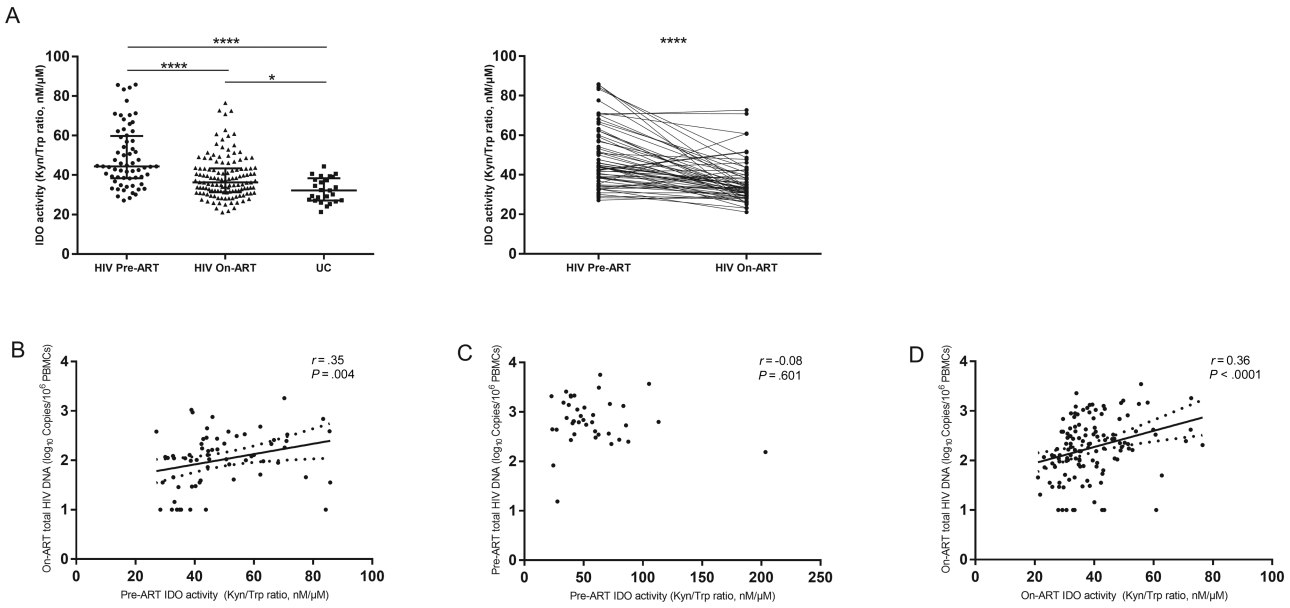

Figure 1. Indoleamine 2,3-dioxygenase (IDO) activity and its association with total human immunodeficiency virus (HIV) DNA. *A*, Elevated IDO activity in HIV-infected patients and longitudinal changes in IDO activity pre–antiretroviral therapy (ART) and on-ART. Pre-ART IDO activity predicted on-ART HIV DNA (*B*) but was not associated with total pre-ART HIV DNA (*C*). On-ART IDO activity was positively correlated with on-ART HIV DNA (*D*). *$P < .05$, ****$P < .0001$. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IDO, indoleamine 2,3-dioxygenase; Kyn/Trp, kynurenine/tryptophan; PBMC, peripheral blood mononuclear cell; UC, human immunodeficiency virus–uninfected controls.

T-cell counts <200 cells/μL (r = 0.51, P = .011; Table 2). After adjustment for other factors that were associated with on-ART HIV DNA, low pre-ART CD4/CD8 ratio and high on-ART IDO activity were independently associated with high on-ART HIV DNA (higher than the median level) in a multivariate binary logistic regression model (adjusted odds ratio [aOR], 0.75 per 0.1 increase [95% confidence interval {CI}, .62–.91]; aOR, 1.09 per nM/μM increase [95% CI, 1.04–1.14], respectively; Table 3).

Microbial Translocation Persists on ART but Was Not Associated With HIV Reservoir Size

Plasma levels of sCD14 were elevated while LPS and LBP were comparable and EndoCAb was lower among untreated participants compared with HIV-uninfected controls (Figure 2A and Table 2). However, none of these markers predicted on-ART HIV DNA (Figure 2B). After effective ART, plasma EndoCAb increased to a level similar to that of HIV-uninfected controls.

Table 3. Factors Associated With High Total Human Immunodeficiency Virus DNA on Antiretroviral Therapy

	Univariate Logistic Regression			Multivariate Logistic Regression		
Factor	OR	(95% CI)	*P* Value	OR	(95% CI)	*P* Value
Age[a]	1.00	(.97–1.03)	.994			
Male sex	1.02	(.28–3.70)	.979			
CD4 T-cell count[b]	0.74	(.59–.93)	.011			
CD8 T-cell count[b]	1.07	(.99–1.16)	.075			
CD4/CD8 ratio[c]	0.75	(.63–.90)	.002	0.75	(.62–.91)	.003
HIV RNA[d]	1.46	(.80–2.69)	.215			
ART duration[e]	0.92	(.88–.97)	.001			
On-ART CD4 T-cell count[b]	0.77	(.62–.95)	.015			
On-ART CD8 T-cell count[b]	1.17	(1.04–1.32)	.011			
On-ART CD4/CD8 ratio[c]	0.80	(.71–.91)	.001			
On-ART IDO activity[f]	1.09	(1.04–1.13)	<.0001	1.09	(1.04–1.14)	<.0001

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; IDO, indoleamine 2,3-dioxygenase; OR, odds ratio.

[a]Shown per 1 year increase.

[b]Shown per 100 cells/μL increase.

[c]Shown per 0.1 increase.

[d]Shown per 1 $\log_{10}$ copies/mL increase, data from 99 patients.

[e]Shown per 1 month increase.

[f]Shown per 1 nM/μM increase.

Levels of on-ART plasma sCD14 were still higher than those in HIV-uninfected controls. There was no correlation between on-ART HIV DNA and any of these plasma markers among treated participants (Figure 2C).

Plasma levels of sCD163 were elevated in untreated participants, and decreased but did not normalize on ART (Figure 2D). The pre-ART level of plasma sCD163 was not associated with on-ART HIV DNA, whereas the on-ART level of sCD163 almost positively correlated with HIV DNA ($r = 0.11$, $P = .398$ and $r = 0.27$, $P = .033$, $q = .072$, respectively; Figure 2D).

T-Cell Activation and CD4 T-Cell Exhaustion Were Positively Correlated With HIV DNA

The proportions of HLA-DR$^+$ CD38$^+$ cells in both CD4 and CD8 T-cell compartments marginally correlated with total HIV DNA levels in treated participants ($r = 0.31$, $P = .028$, $q = .065$ and $r = 0.32$, $P = .026$, $q = .065$, respectively; Figure 3A). In addition, the proportion of PD-1–expressing CD4 T cells but not PD-1$^+$ CD8 T cells marginally significantly correlated with HIV DNA levels ($r = 0.31$, $P = .026$, $q = .065$ and $r = 0\ .11$, $P = .455$, respectively; Figure 3A). Proportions of HLA-DR$^+$CD38$^+$ CD4 T cells, HLA-DR$^+$CD38$^+$ CD8 T cells, PD-1$^+$ CD4 T cells, and PD-1$^+$ CD8 T cells did not correlate with concurrent IDO activity (Figure 3B).

DISCUSSION

Identification of factors that are linked with the size of HIV reservoir is essential to HIV eradication. In the current study, we assessed associations between IDO activity, microbial translocation, immune activation, T-cell exhaustion, and total HIV DNA in peripheral blood in a cohort of well-treated HIV-infected patients. We observed a positive correlation of total HIV DNA with IDO activity, with immune activation as well as with T-cell exhaustion, but not with microbial translocation.

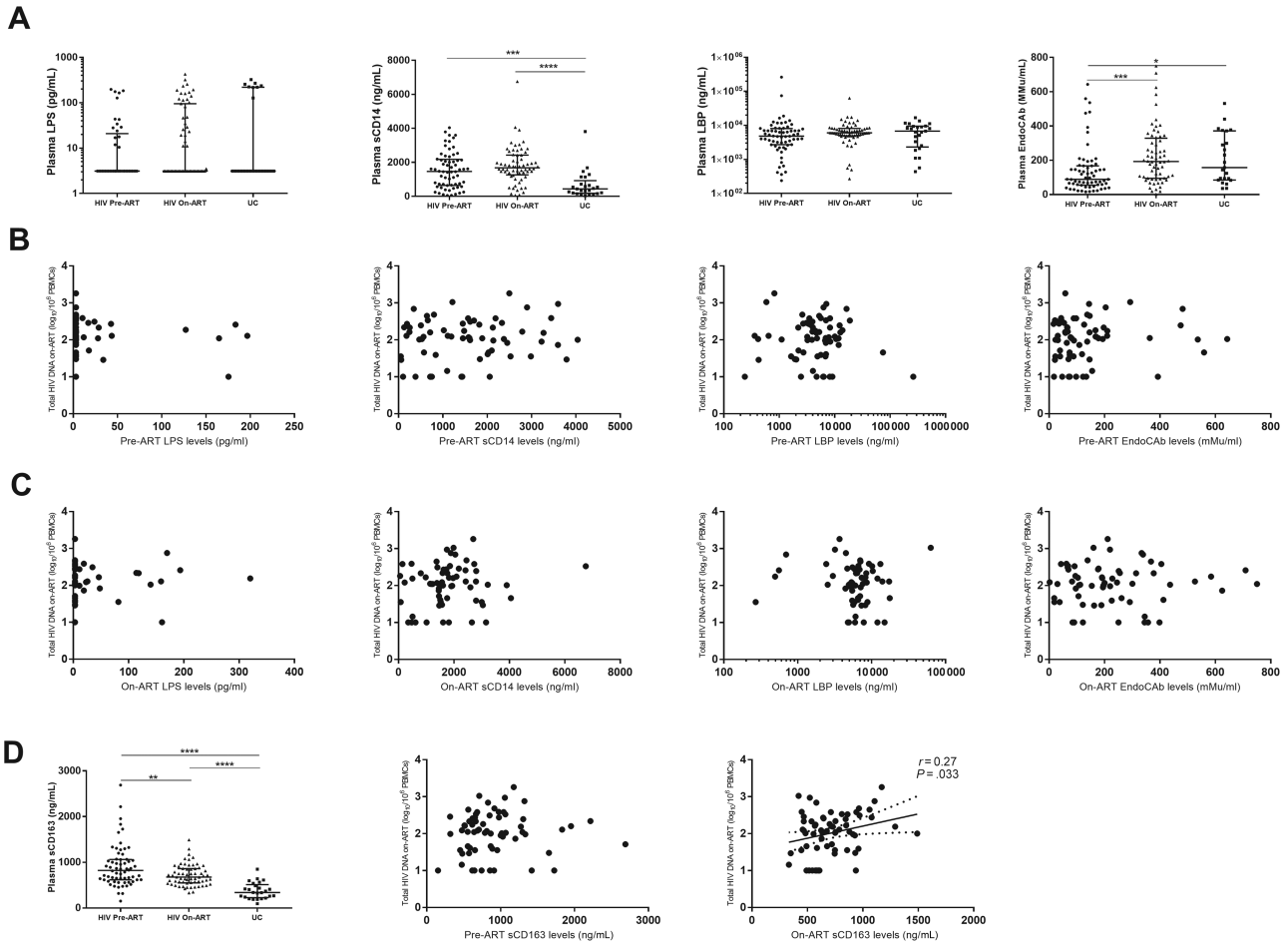

Figure 2. No association between microbial translocation and human immunodeficiency virus (HIV) persistence. *A*, Changes of microbial translocation markers. *B*, Pre–antiretroviral therapy (ART) levels of microbial translocation could not predict on-ART total HIV DNA. *C*, Microbial translocation markers were not associated with on-ART total HIV DNA. *D*, On-ART but not pre-ART sCD163 was associated with on-ART total HIV DNA. Microbial translocation markers were quantified in 64 patients with paired samples available both before and after ART. *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$. Abbreviations: ART, antiretroviral therapy; EndoCAb, endogenous endotoxin-core antibody; HIV, human immunodeficiency virus; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; sCD14, soluble CD14; sCD163, soluble CD163; UC, human immunodeficiency virus–uninfected controls.

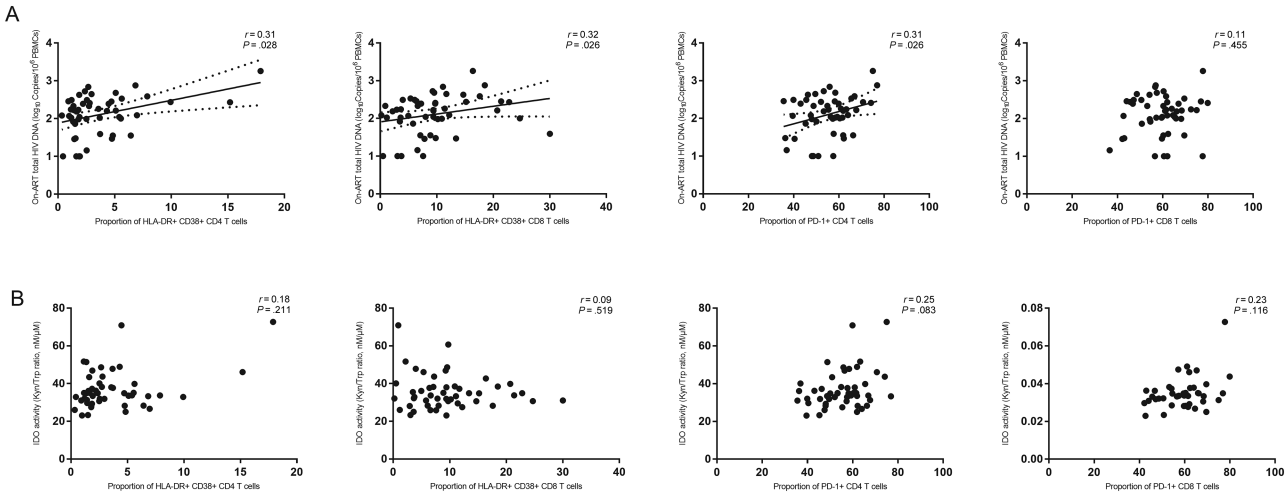

Figure 3. T-cell activation and exhaustion were associated with human immunodeficiency virus (HIV) persistence (*A*) but not indoleamine 2,3-dioxygenase activity (*B*). HLA-DR, CD38, and PD-1 were assessed in samples from 50 of the participants. Abbreviations: ART, antiretroviral therapy; CD38, cluster of differentiation 38; HIV, human immunodeficiency virus; HLA-DR, human leukocyte antigen – DR isotype; IDO, indoleamine 2,3-dioxygenase; Kyn/Trp, kynurenine/tryptophan; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death 1.

To the best of our knowledge, this is the first study to report a correlation between IDO activity and total HIV DNA. During HIV infection, IDO activity has been attributed to pathogenesis. Elevated IDO activity has been associated with HIV disease progression, Kaposi sarcoma, active tuberculosis, poor CD4 T-cell recovery, cardiovascular disease, HIV-associated neurocognitive disorder, and mortality [12, 16, 25]. Alongside the persistence of HIV reservoirs on long-term suppressive ART, the elevated IDO activity is also not normalized even after >7 years of ART [11, 12, 14]. Interestingly, IDO activity was associated with total HIV DNA in our study and not with microbial translocation and immune activation. Along these lines, it is reasonable to speculate that the elevated IDO activity is driven by the persistent HIV infection or vice versa, suggesting it may be one of the strategies that HIV uses to escape eradication. Clonal expansion of the latently infected memory CD4 T cells, which can be driven by foreign antigens and cytokines, have been attributed to HIV persistence [19]. Recent studies suggested that clonal expansion may be driven by antigens from tumors and pathogens (eg, cytomegalovirus and Epstein-Barr virus) [26, 27]. Given the immunosuppressive role of IDO, elevated IDO activity may impede the clearance of these antigens, which in turn expand the HIV reservoir [28]. Although it is still controversial, another mechanism for HIV persistence involves ongoing low-level viral replication on ART, especially in anatomical sites such as lymph nodes where drug concentrations are insufficient to completely suppress HIV replication [1, 29, 30]. Owing to its immunosuppressive role, it is also possible that the up-regulated IDO activity in these sites facilitates HIV replication. In fact, IDO and its downstream kynurenine pathway play a critical role in maintaining immune privilege in brain, eyes, and testes, which are also sites of HIV persistence during suppressive ART [28]. Testes from HIV-infected patients show elevated IDO activity with an increase in the frequency of regulatory T cells as compared to blood, which may facilitate HIV persistence [31]. In an animal model of HIV-1 encephalitis, inhibition of IDO has been shown to enhance elimination of virus-infected macrophages [32]. Combined ART and blockade of IDO has also been shown to reduce the viral load significantly in simian immunodeficiency virus–infected rhesus macaques with unsuccessful ART [33]. Taken together, IDO activity may be involved in HIV persistence and might be a potential immunotherapeutic target to reduce HIV reservoir size. Therefore, further research, both in vitro and in vivo, needs to be done to examine the effects of IDO inhibitors on reducing the size of HIV reservoir.

In contrast to on-ART IDO activity, no association between pre-ART IDO activity and pre-ART total HIV DNA was observed. This can be explained by factors that influence IDO activity, for example, pre-ART microbial translocation and HIV RNA levels. However, it may also be attributed to the different distribution of the integrated HIV, as HIV DNA is more likely to be detected in active CD4 T cells in untreated participants and more in resting T cells after ART initiation [1].

Expression of PD-1 on T cells has been previously associated with HIV persistence on ART, which we also observed in this study [17, 19, 34]. PD-1 is an immune checkpoint marker that suppresses immune responses, high expression of which on T cells represents an immunomodulatory microenvironment that may contribute to HIV persistence. Our study did not find evidence to support this mechanism as no significant association between proportion of PD-1$^+$ CD8 T cells and total HIV DNA was observed, which is in line with previous studies that assessed blood and gut tissues [18, 35]. However, a recent study reported correlation between CD8

T-cell exhaustion and integrated HIV DNA in lymph nodes [35]. Blockade of PD-1 also leads to reversal of HIV latency [36]. On the other hand, PD-1 itself could be a potential surrogate marker of HIV reservoir. In HIV-infected individuals, central and transitional memory CD4 T cells that express high levels of PD-1 have higher HIV DNA than those cells that express low levels of PD-1 [19, 34]. In lymph nodes, CD4 T cells expressing PD-1, which are mainly T follicular helper cells, are the major source of replication-competent HIV-1 [37]. In this context, blocking PD-1 might be a promising strategy to decrease HIV reservoir size. Interestingly, a case with drastic and sustained decrease of the HIV reservoir under anti–PD-1 therapy has been recently reported, although its reproducibility needs to be established [38].

In our study, the proportion of PD-1–expressing T cells was not associated with IDO activity. Interestingly, a landmark study recently revealed that increased IDO activity in tumor-repopulating cells could induce PD-1 expression on T cells [39]. Whether this strategy is adopted by HIV in the microenvironment still needs to be studied. If that is the case, a combination of anti–PD-1/PD-L1 and IDO inhibitors may be more effective than anti–PD-1/PD-L1 alone in eradicating HIV reservoirs. This combination strategy is promising in treating cancers, although a recent clinical trial failed to report additive effects of IDO inhibitor among patients with melanoma receiving anti–PD-1 therapy [36, 40].

Several groups have demonstrated the positive correlation between immune activation and HIV persistence, while others reported the lack of an association [17, 18, 20, 21, 35]. Such inconsistent results may be explained by differences in the study population with variable duration of ART, distinct sites of sample collection, and different methods for HIV quantification used. We observed that persistent microbial translocation after ART does not correlate, or at least does not directly contribute to HIV persistence, as we failed to find any association between microbial translocation markers and total HIV DNA.

Our results need to be interpreted with caution owing to some study limitations. First, we quantified HIV persistence and tryptophan and kynurenine concentrations in peripheral blood, which may not represent the microenvironments in tissues, where a significant portion of HIV reservoir is located. Another limitation is the use of PCR-based methods to measure HIV persistence, which could overestimate the HIV reservoir size as most of the viral genomes detected are defective or replication incompetent [2]. Third, IDO activity was measured at pre-ART and on-ART time points in 64 of 127 participants owing to the limited availability of samples. Finally, our study did not enroll patients with long-term (eg, >10 years) successive ART. The decay slopes of HIV reservoir among patients on variable durations of ART are different, which is greatest during the first 4 years of ART [21]. Additional studies of populations with such attributes are therefore required to increase the generalizability of the findings.

In summary, our study found a positive correlation between IDO activity and total HIV DNA in blood, suggesting its involvement in HIV persistence. Further studies are warranted to clarify underlying mechanisms and explore whether interventions targeting this pathway may help reduce HIV reservoir size.

Supplementary Data

Supplementary materials are available at *Clinical 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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