Nonstructured Treatment Interruptions Are Associated With Higher Human Immunodeficiency Virus Reservoir Size Measured by Intact Proviral DNA Assay in People Who Inject Drugs

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The latent reservoir for human immunodeficiency virus type 1 (HIV-1) in CD4⁺ T cells is a major barrier to cure. HIV-1-infected persons who inject drugs (PWID) often struggle to maintain suppression of viremia and experience nonstructured treatment interruptions (NTIs). The effects of injecting drugs or NTIs on the reservoir are unclear. Using the intact proviral DNA assay, we found no apparent effect of heroin or cocaine use on reservoir size. However, we found significantly larger reservoirs in those with frequent NTIs or a shorter interval from last detectable HIV RNA measurement. These results have important implications for inclusion of PWID in HIV-1 cure studies.

Keywords. HIV-1 latent reservoir; HIV-1 viral suppression; people who inject drugs; PWID; antiretroviral therapy; ART; nonstructured treatment interruption; NTI.

The latent reservoir for human immunodeficiency virus type 1 (HIV-1) in resting CD4⁺ T cells is a major barrier to cure [1–8]. It is comprised of latently infected resting CD4⁺ T cells carrying integrated, replication-competent viral genomes that are not expressed unless cells are activated. The reservoir has not been extensively studied in persons who inject drugs (PWID). In mixed cohorts with various modes of acquisition, the frequency of latently infected cells as detected by the quantitative viral outgrowth assay (QVOA) varies over a 2-log range centered around 1 infectious unit per million (IUPM) CD4⁺ T cells [3, 5, 7–9]. The size of the reservoir in PWID likely falls in this range, but whether there are major differences relative to other populations of infected individuals is unclear. As cure interventions move into clinical trials [10–12], it is important to understand how injection drug use affects the latent reservoir and the potential for cure.

Drugs such as heroin and cocaine may affect the reservoir directly and indirectly. Increased intensity of active injection drug

use is directly associated with markers of inflammation, independent of the effects of HIV-1 or hepatitis C virus infection [13], and inflammatory mediators could affect both the establishment and maintenance of the reservoir [14]. In addition, a major effect of drug use on the latent reservoir may operate indirectly through drug use-associated problems with adherence to antiretroviral therapy (ART). Many PWID have a history of nonstructured treatment interruption (NTI), with one-third having multiple interruptions, cycling on and off ART almost annually [15].

An additional problem is that the reservoir cannot be accurately measured unless active viral replication is suppressed for >6 months. Following ART initiation, the frequency of resting CD4⁺ T cells harboring inducible, replication-competent HIV-1 genomes as detected by the QVOA falls down to a steady state level of approximately 1 IUPM over 6–9 months [16]. Prior to that, the small pool of cells that constitute the stable latent reservoir is masked by a much larger pool of recently infected cells that carry replication-competent HIV-1 genomes but that do not enter the stable reservoir. For this reason, the reservoir is generally measured only after >6 months of suppressive ART. This complicates reservoir analysis in individuals with frequent NTIs.

The QVOA is the gold standard assay for the latent reservoir, but it requires large blood volumes, which can be problematic in PWID. In addition, it requires an in vitro stimulation to reverse latency and allow viral outgrowth [3–5, 7–9]. Recent studies have shown that not all replication-competent

Received 17 July 2020; editorial decision 29 September 2020; accepted 2 October 2020; published online October 10, 2020.

Presented in part: Virtual Conference on Retroviruses and Opportunistic Infections, March 2020.

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The Journal of Infectious Diseases® 2021;223:1905–13

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DOI: 10.1093/infdis/jiaa634

proviruses are induced by a single round of in vitro T-cell activation [17–19]. Thus, the QVOA may underestimate reservoir size. Simpler approaches to reservoir measurement involving subgenomic polymerase chain reaction (PCR) assays to detect proviral DNA are also problematic because a large fraction of proviruses are defective as result of large deletions and/or APOBEC3G-mediated hypermutation [9, 17, 20–24]. Hence, standard DNA PCR assays dramatically overestimate reservoir size by including defective proviruses that cannot contribute to viral rebound. The recently described intact proviral DNA assay (IPDA) separately quantitates intact and defective proviruses [21, 25, 26] and has been used to show that these 2 populations of proviruses have different in vivo decay rates [21, 27, 28]. The IPDA may therefore provide the best way to assess the reservoir in PWID.

To evaluate the effects of heroin and cocaine use on the latent reservoir, we used the IPDA to analyze samples from the AIDS Linked to the IntraVenous Experience (ALIVE) cohort of PWID [29]. The large size and high retention rates of this cohort coupled with an extensive longitudinal database linked to a biorepository allowed us to identify participants who had suppression of viremia on ART to below the limit of

detection in 2 consecutive study visits 6 months apart [30]. We analyzed the size of the reservoir in 120 samples from 99 study participants who met these criteria and had 4 different patterns of drug use reported over the prior 6 months: no current drug use, heroin use, cocaine use, or use of both drugs. The results provide new insights into factors affecting reservoir size and the potential for cure in PWID.

METHODS

Study Participants and Samples

ALIVE participants have study visits every 6 months for collection of drug use data and cryopreservation of peripheral blood mononuclear cell (PBMC) samples. Because stable suppression of viremia for ≥ 6 months is required for accurate reservoir analysis [16], we identified participants who had plasma HIV-1 RNA levels below the limit of detection on 2 consecutive study visits and analyzed samples from the second of these visits (Figure 1A). From participants meeting these criteria, we selected 4 balanced sets of 30 samples based on self-reported patterns of drug use at the sampled visit: no active illicit drug use, active heroin use only, active cocaine use only, and active use of both heroin and cocaine. For some participants, these

Figure 1. IPDA analysis of the latent reservoir in PWID. **A**, Study design. HIV-1 RNA values from ALIVE study participants at biannual study visits were analyzed to identify participants with 2 consecutive values below the limit of detection, suggestive of stable suppression of viremia on ART. Equal numbers of participants with 4 different patterns of self-reported drug use at the time of the second of these visits were selected. Drug use groups were also balanced with respect to frequency of “switches” in HIV-1 RNA status (detectable vs undetectable, see Methods). Samples from the second visit were used for measurement of intact proviruses by IPDA. In the IPDA, DNA from purified CD4⁺ T cells is distributed into nanoliter sized droplets so that individual HIV-1 proviruses (red bars) can be interrogated with duplex PCRs (blue and green arrow) for deletions (white) and hypermutation. Intact proviruses lacking large deletions and hypermutation are directly counted as double positive droplets in the upper right quadrant. See reference [21] for details. **B**, IPDA values for intact proviruses, proviruses with 3' defects (deletions and/or hypermutation), proviruses with 5' defects (deletions), and total detected proviruses for all samples ($N = 108$). Values are expressed as the number of proviruses/ 10^6 CD4⁺ T cells. Boxes, middle quartiles. Horizontal lines within boxes, median value. Open circles represent samples for which no intact proviruses were detected among the cells analyzed. For these samples, a maximum value was estimated as 10^6 /(number of cells analyzed). For each of these samples, proviruses with 3' defects and proviruses with 5' defects were detected. Abbreviations: HIV-1, human immunodeficiency virus type 1; IPDA, intact proviral DNA assay.

criteria were met for >1 pair of study visits. An attempt was made to balance each group with respect to 1 additional parameter, the number of previous NTIs as defined by switches between detectable and undetectable HIV-1 RNA values on consecutive study visits (Figure 1A). Within each drug use group, participants with more frequent NTIs (determined to be viral switch at $\geq 18\%$ of study visits) were selected in a 2:1 ratio to those with less frequent switches. The mean time participants were followed within the ALIVE cohort before sampling was 10.6 years. Some of the earlier samples used in this study were obtained when clinical assays for HIV-1 plasma RNA had a limit of detection of 400 copies/mL. For consistency, we have used this value in the analysis of NTIs. This choice lessens the chance of misclassifying clinically insignificant blips as an indication of treatment interruption [31]. Study participants were mostly positive for hepatitis C infection by serology (93.5%) but negative for hepatitis B surface antigen (98.1%). The Johns Hopkins Bloomberg School of Public Health Institutional Review Board reviewed and provided continuous approval for study procedures, and all participants provided written informed consent.

Intact Proviral DNA Assay

De-identified, previously collected samples blinded as to participant characteristics and drug use history were provided to Acelevir Diagnostics as cryopreserved PBMCs for IPDA analysis. In-depth descriptions of the IPDA rationale and procedure have been previously published [21, 25, 27].

RESULTS

Study Participants

We analyzed a total of 120 samples from 99 unique individuals (Table 1). The study population was predominantly male (72.6%) and African American (95.6%) with a median age at sampling of 52.8 years (interquartile range [IQR], 49.1–56.8 years). Most participants were unemployed (91.0%), but few had experienced incarceration (7.0%) or homelessness (5.5%) during the previous 6 months. The study population had a median CD4 count of 448 cells/ μ L at the time of sampling and a median CD4 nadir of 173 cells/ μ L. Relative to the sample time point, the median time since first ART was 7.8 years. However, median time since the last detectable HIV-1 RNA was estimated at 3.4 years, and 67.0% of the cohort had HIV-1 RNA switches

Table 1. Characteristics of Study Participants

Characteristic	Samples Analyzed				
	Entire Cohort	Group 1 (None)	Group 2 (Heroin)	Group 3 (Cocaine)	Group 4 (Both)
	108 ^a	28	28	23	29
General					
Unique participants	91 ^a	28	28	23	29
Male sex, %	72.6	67.9	71.4	65.2	79.3
African American race, %	95.6	92.9	92.9	100.00	96.6
Age ^b , y, median (IQR)	52.8 (49.1–56.8) ^c	52.6 (46.7–56.6)	53.1 (49.5–56.7)	51.2 (49.4–56.9)	56.0 (50.9–59.8)
Social, %					
Employed ^d	9.0 ^c	10.7	7.4	9.1	3.4
Incarceration ^d (>1 wk)	7.0 ^c	0.0	0.0	0.0	17.2
Homelessness ^d (any)	5.5 ^c	0.0	7.1	8.7	6.9
Alcohol use ^d	49.5 ^c	14.3	67.9	47.8	58.6
Tobacco use ^d	78.0 ^c	78.6	75.0	82.6	86.2
Injection drug use ^d	28.6 ^c	0.0	28.6 ^e	26.1	70.0
Clinical					
Proximal CD4 ^b count, cells/ μ L, median	448 ^c	408	464	482.0	432
CD4 nadir, cells/ μ L	173	137	183.5	220	188
Time from first ART, y	7.8	10.6	7.6	6.7	7.4
Time from last positive HIV-1 VL ^b , y	3.40	3.19	4.07	2.49	4.14
Proportion with high switch, % ^{b,f}	67.0	64.3	64.3	73.9	62.1

Abbreviations: ART, antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; VL, viral load.

^aA total of 99 participants provided 120 samples. Twenty participants were sampled at 2 time points, and 1 participant was sampled at 3 time points. Of the 120 samples, 7 samples from 5 different participants were excluded due to intact proviral DNA assay signal failure, and 5 samples from 5 different participants were excluded due to instrument failure, resulting in a final set of 108 samples for analysis from 91 unique donors.

^bAt the time of sampling, except where indicated.

^cAt the first sample time point for participants sampled more than once.

^dDuring the 6 months prior to sampling.

^eHeroin use by other than injection accounts for the low fraction of participants in the heroin use group reporting intravenous injection.

^fPercentage of participants experiencing a high number of switches in HIV-1 RNA status. A switch was defined as a change in HIV-1 RNA from detectable to undetectable (or undetectable to detectable) relative to the previous study visit. Switches were considered high if a switch was seen at $\geq 18\%$ of study visits (10% for the heroin-only group due to lower numbers).

at $\geq 18\%$ of study visits. These parameters were well balanced between the 4 drug use groups (Table 1).

IPDA Analysis

The IPDA directly quantifies intact proviruses, defined here as proviruses lacking the 2 most common types of fatal defects: large deletions and APOBEC3G-mediated hypermutation [21]. DNA from purified CD4⁺ T cells is dispersed into thousands of nanoliter-sized droplets so that individual proviruses can be interrogated at 2 informative positions using duplex PCR reactions occurring within the droplets (Figure 1A). Amplicon positions are chosen so the vast majority of defective proviruses will fail to give amplification at both positions, and thus intact proviruses can be directly counted as double positive droplets [21]. A separate digital droplet PCR assay measures input cellular DNA and allows correction for DNA shearing [21].

IPDA analysis was carried out on CD4⁺ T cells purified from cryopreserved PBMC from the sampling time point as shown in Figure 1A. For 5 of 99 participant samples (5.1%), there was signal failure for 1 of the IPDA amplicons. As previously described [25, 27], sequence polymorphism can cause amplification issues, precluding the measurement of intact proviruses without alternative primers or probes. All samples ($n = 7$) from these participants were excluded from the analysis, as were 5 samples for which instrument malfunction precluded measurement. For the remaining samples ($n = 108$), the median frequency of intact proviruses was 175 per 10^6 CD4⁺ T cells (IQR, 29–345). Notably, these values are similar to those recently reported for a distinct cohort of HIV-1–infected adults in whom injection drug use was rare (151 per 10^6 CD4⁺ T cells [IQR, 40–398] [27]). For 13 of 108 samples (12%), no intact proviruses were detected in the cells analyzed, suggesting a low reservoir size (Figure 1B). Both 3′ and 5′ defective proviruses were detected in all of these samples, excluding amplicon failure as a cause of the absence of intact proviruses. As with most reservoir measurements [8, 9, 25, 32], intact provirus values varied over a 2- to 3-log range in different individuals (Figure 1B). Consistent with previous studies [21, 25], the frequency of cells with intact proviruses was significantly lower than the frequency of cells with defective proviruses (median values of 576 and 357 per 10^6 CD4⁺ T cells for 3′ defective and 5′ defective proviruses, respectively; Figure 1B). Based on median values, intact proviruses represent only 13% of total detected proviruses (Figure 1B), highlighting the importance of selective measurement of intact proviruses. Overall, these results suggest that the HIV-1 reservoir is not substantially larger in PWID than in other infected individuals.

Effect of Current Drug Use

To assess the effects of ongoing use of heroin and/or cocaine on reservoir size, we compared the frequency of CD4⁺ T cells with intact proviruses in 4 groups with different patterns of

self-reported drug use (Figure 2A). In participants denying active use of heroin or cocaine but with a history of injection drug use, the median frequency of cells with intact proviruses was 90 per 10^6 CD4⁺ T cells (IQR, 12–306). Median intact proviral levels for study participants in the other drug use groups were also in this range (heroin: 199 per 10^6 CD4⁺ T cells [IQR 31–307]; cocaine: 277 per 10^6 CD4⁺ T cells [IQR, 42–502]; both: 105 per 10^6 CD4⁺ T cells [IQR, 23–316]). The differences in intact provirus levels between the groups were not significant (Figure 2A). In addition, levels of proviruses with defects at the 3′ or 5′ end of the genome were also similar among the drug use groups (Supplementary Figure 1). Together these results show that neither a history of injection drug use nor self-reported active use of heroin and/or cocaine has a major effect on the size of the latent reservoir in PWID on suppressive ART.

Effect of NTIs

Drug addiction is associated with problems with adherence and engagement in care, with some PWID experiencing repeated NTIs [15, 30]. To determine whether NTIs affect reservoir size, we determined the number of switches in plasma HIV-1 RNA from undetectable to detectable (or detectable to undetectable) on consecutive study visits for each participant over the course of follow-up (median, 10.6 years). We then compared intact provirus values in participants who had relatively stable suppression of viremia on ART (“low switches”) to values in participants with frequent switches (“high switches,” defined as switches on $>18\%$ of biannual study visits). As shown in Figure 2B, high switch participants had significantly higher levels of intact proviruses than low switch participants (median, 249 vs 43 per 10^6 CD4⁺ T cells, respectively; $P = .0004$).

Effect of Time Since Last Detectable HIV-1 RNA Level

As part of the study design, all participants had HIV-1 RNA levels below the limit of detection at the time of sampling and at the study visit 6 months previously (Figure 1A). However, some participants had maintained suppression of viremia on ART for many years, whereas for other participants, particularly those with frequent NTIs, the period of sustained virologic suppression was much shorter. The median time since last detectable viral load for the overall study population was 3.4 years (IQR, 1.6–6.1 years). As shown in Figure 2C, levels of intact provirus declined with increasing time since last detectable HIV-1 RNA level. After exploring multiple cutpoints, we dichotomized years since last detectable HIV-1 RNA at 3 years for further analysis. Participants with <3 years compared to those with ≥ 3 years since last detectable viral load had significantly higher levels of intact proviruses (median, 282 vs 80 per 10^6 CD4⁺ T cells, respectively; $P = .0004$).

Figure 2. Effect of current patterns of self-reported drug use and previous NTIs on the size of the latent reservoir. *A*, Intact provirus levels in PWID reporting 4 different patterns of heroin and cocaine use at the time of sampling. Boxes, middle quartiles. Horizontal lines within boxes, median value. Open symbols, no intact proviruses detected - the plotted value = 106/(cells analyzed). Significance of differences between groups was determined by two-tailed T-test on log10-transformed values. *B*, Intact provirus levels in PWID with a low or high fraction of switches in HIV-1 RNA detectability on consecutive study visits. *C*, Relationship between intact provirus levels and time since last + HIV-1 RNA measurement. Solid line, regression line for log10 (intact provirus level) vs. time. Red and blue lines are separate regression lines for High and Low switch participants, respectively. Dotted line, exponential reservoir decay for $t_{1/2} = 3.67$ yrs. *D*, Estimated initial intact provirus levels assuming exponential decay at $t_{1/2} = 3.67$ yrs for the period of sustained suppression prior to sampling, stratified by history of a low or high fraction of switches in HIV-1 RNA detectability. Abbreviation: HIV, human immunodeficiency virus.

Correlates of Intact Provirus Levels

To understand the relationship between NTIs, duration of viral suppression, and size of the latent reservoir, we analyzed correlates of intact provirus levels in univariate and multivariable regression models. In univariate analysis (Table 2), we found no association with pattern of self-reported drug use. However, age ≥ 50 years and female sex were both associated with lower intact provirus levels. In addition to these demographic variables, both higher switch frequency and shorter time since last detectable HIV-1 viral load were significantly associated with higher intact provirus levels (Table 2). These 2 variables are related (Figure 2C); a higher proportion of viral switches was strongly correlated with shorter time since last detectable HIV-1 viral load (higher switches: median of 2.1 years [IQR, 1.5–4.1 years]; lower switches: 5.0 years [IQR, 4.3–8.7 years]). In separate multivariable models adjusting for demographics and drug use groups, higher switching ($P = .0032$) and < 3 years from the last detectable HIV-1 viral load ($P = .0087$) were each significantly and independently correlated with higher intact provirus levels (Table 2, models

A and B, respectively). Intact and total provirus levels are highly correlated (Spearman $\rho = 0.76$; $P < .0001$ [21]). Thus, associations with higher switching and < 3 years from the last detectable HIV-1 viral load were also seen using the log of total proviruses as the dependent variable ($P = .0009$ and $P = .0098$, respectively). In a final model adjusting for demographics and drug use groups (Table 2, model C), we incorporated an interaction term representing joint exposure to both of these variables (high switching alone, < 3 years from last detectable viral load alone, and both high switching and < 3 years from last detectable viral load). These were compared to low switching and ≥ 3 years from last detectable viral load as the reference group. In this multivariable model, we observed a dose-response association of intact provirus levels increasing with joint exposure. Of note, although the point estimate suggested substantial increase in intact provirus levels, there were only 4 samples from participants with a low proportion of switching and a short interval since last detectable viral load. As a result, this comparison was not statistically significant. Overall, these results suggest that frequent NTIs and a short

Table 2. Correlates of Intact Provirus Levels

Characteristic	Univariate		Model A ^a		Model B ^a		Model C ^b	
	Estimates (95% CI)	PValue	Estimates (95% CI)	PValue	Estimates (95% CI)	PValue	Estimates (95% CI)	PValue
Age >50 y	−0.58 (−.97 to −.19)	.0038	−0.67 (−1.04 to −.29)	.0006	−0.62 (−1.00 to −.24)	.0017	−0.64 (−1.00 to −.24)	.0010
Female sex	−0.41 (−.83 to −.00)	.0489	−0.47 (−.86 to −.08)	.0191	−0.49 (−.88 to −.09)	.0165	−0.42 (−.88 to −.09)	.0384
Black race	0.67 (−.23 to 1.56)	.1436	0.54 (−.28 to 1.36)	.1927	0.47 (−.36 to 1.30)	.2628	0.48 (−.36 to 1.30)	.2524
Drug use group ^c								
None	1.00		1.00		1.00		1.00	
Heroin only	0.29 (−.24 to .82)	.2816	0.32 (−.15 to .79)	.1845	0.37 (−.11 to .85)	.1320	0.33 (−.11 to .85)	.1707
Cocaine only	0.38 (−.18 to .94)	.1788	0.30 (−.20 to .80)	.2385	0.29 (−.22 to .80)	.2611	0.27 (−.22 to .80)	.2832
Cocaine and heroin	0.18 (−.34 to .71)	.4957	0.20 (−.27 to .68)	.4037	0.24 (−.24 to .72)	.3194	0.24 (−.24 to .72)	.3226
Injection drug use frequency ^c			...		...		...	
None	1.00							
<1 time per day	−0.39 (−.89 to .12)	.1342						
Daily	−0.10 (−.65 to .46)	.7313						
Lower switching ^d	1.00		1.00		...		...	
Higher switching	0.65 (.27–1.03)	.0011	0.56 (.19–.93)	.0032	...		...	
Years with UVL ^e								
≥3	1.00		...		1.00		...	
<3	0.62 (.25–.98)	.0011	...		0.49 (.13–.85)	.0087	...	
Low switch, UVL ≥3 y	...		...		...		1.00	
High switch, UVL ≥3 y	...		...		...		0.49 (.02–.96)	.0396
Low switch, UVL <3 y	...		...		...		0.59 (−.38 to 1.56)	.2280
High switch, UVL <3 y	...		...		...		0.73 (.31–1.16)	.0009

Abbreviations: CI, confidence interval; UVL, undetectable viral load.

^aMultivariable model adjusting for demographics and drug use groups.^bMultivariable model adjusting for demographics and drug use groups and incorporating an interaction term representing joint exposure to high switching and <3 years of UVL.^cBased on self-report at sample time point.^dBased on fraction of study visits at which there was a switch in human immunodeficiency virus type 1 RNA suppression status (eg, from undetectable to detectable, or detectable to undetectable). Higher switching is defined as a switch at >18% of study visits. Participants were followed biannually for a median of 10.6 years.^eYears with UVL. Calculated as years from last detectable viral load prior to sample visit.

time of sustained suppression of viremia on ART can both contribute to higher levels of intact proviruses.

Effect of Reservoir Decay

Given that NTIs and shorter time since the last detectable viral load were both associated with higher intact provirus levels, we hypothesized that frequent NTIs might simply shorten the period of sustained viral suppression during which reservoir decay could occur. Most of the high switch participants had shorter periods of sustained suppression prior to sampling (Figure 2C). The slope (m) of the regression line for the correlation between the log of the intact provirus levels and time since last positive viral load ($m = -0.090$) was similar to the expected decay slope of the latent reservoir (half-life [$t_{1/2}$] = 3.67 years; $m = -0.078$ [7, 8]). To determine whether the apparent effect of NTIs on intact provirus levels was due solely to shorter time for reservoir decay, we calculated the expected intact provirus level at the time stable suppression of viremia was first achieved, assuming uniform exponential reservoir decay (Figure 2D). After adjustment for reservoir decay, a history of NTIs was still associated with higher estimated initial intact provirus levels ($P = .0447$). Comparison of the slopes of the regression lines for high and low switch participants suggested slower decay for high switch participants (Figure 2C); thus, the higher levels of intact proviruses seen in participants with frequent NTIs might reflect increased deposition of virus in the reservoir during NTIs and decreased reservoir decay, as well as shorter times of sustained suppression during which decay can occur. For participants sampled longitudinally with more than a year and no NTIs between sampling ($n = 10$ for high switch participants, 5 for low switch participants), we also found a slower mean decay rate for high switch participants ($0.0691 \pm 0.237 \text{ year}^{-1}$ vs $0.0997 \pm 0.0957 \text{ year}^{-1}$ for low switch participants). These rates are equivalent to half-lives of 10.03 years vs 6.95 years for high and low switch participants, respectively. These rates are slower than the mean decay rate observed in cohorts not selected for a history of injection drug use (0.189 year^{-1} ; $t_{1/2} = 3.67$ years [7, 8]). Further study is needed to understand the mechanisms underlying the observed effect of NTIs and injection drug use on reservoir size.

DISCUSSION

We examined latent reservoir size among PWID using a novel assay that selectively detects the intact proviruses that cause rebound upon ART interruption. In PWID with current suppression of viremia on ART, we found no apparent effect of active heroin and/or cocaine use on reservoir size as measured by IPDA and no marked difference in reservoir size relative to other cohorts of infected people. However, we found notably larger reservoirs in PWID with past or recent periods of viremia due to NTIs compared to PWID with more stable suppression

of viremia. Further, our data suggest that NTIs contribute to reservoir size directly and do not simply reflect shorter times for reservoir decay.

Our data have important implications for the field. First, they support the inclusion of PWID with stable suppression of viremia on ART in cure studies. Second, they demonstrate that a history of viremia due to NTIs may have lasting effects on the size of the reservoir, and as such, virologic history should be considered when designing or analyzing HIV-1 cure studies. We and others have clearly documented how illicit drug use, particularly the intensity of injecting drugs, is directly associated with the inability of PWID to maintain durable HIV-1 viral suppression [15, 30, 33]. Our data indicate that injecting drugs is more likely to impact latent reservoir size through behavioral mechanisms resulting in repeated loss of viral suppression in contrast to biological effects of the illicit drugs themselves.

Both the accumulation of episodes of virologic failure through NTIs and a shorter duration of sustained viral suppression appear to directly contribute to reservoir size. Through direct analysis of multivariable models incorporating both of these variables, as well as back calculation of initial intact provirus levels at time of initial viral suppression, we show that the effect of NTIs on increasing latent reservoir size does not appear to be solely related to duration of viral suppression. This finding suggests that repeated episodes of HIV-1 viral rebound may result in larger latent reservoirs, potentially making eradication more difficult in individuals with this history. Of note, brief structured treatment interruptions done as part of carefully controlled clinical studies do not appear to increase reservoir size [34, 35]. Our results suggest that further investigation of the mechanisms by which viral rebound contributes to the latent reservoir are needed and that a history of frequent NTIs should be considered in the evaluation of participants in cure studies.

In the analysis of factors contributing to reservoir size in PWID, we did not find an association with patterns of self-reported active cocaine or heroin use. In addition to the statistically significant associations with NTIs and short duration of virologic suppression, we did find effects of gender and age. There were lower intact provirus levels in female participants and in participants >50 years of age. Previous studies of gender differences in the HIV-1 reservoir have given conflicting results, likely influenced by the assay used and other factors [26, 36, 37].

This study had several strengths including access to a well-characterized longitudinal PWID cohort linked to biorepository samples, a novel study design that allowed concurrent assessment of both types of illicit drug use as well as viral suppression switching, and application of a state-of-the-art IPDA assay for reservoir characterization. Although our study was limited to characterization of drug use exposure based on self-report, ongoing work will provide biological measures to validate exposure data.

In summary, the latent reservoir size among PWID is comparable to that of other HIV-1 populations and does not appear to be dependent on type of illicit drug used. Cure studies should be designed to include broadly representative populations of infected individuals; exclusion of PWID with substantial duration of viral suppression is unjustified. Both the use of longer-acting ART and implementation of effective HIV care engagement interventions (eg, substance use treatment, peer navigation) hold promise for prolonging the duration of noninterrupted viral suppression among PWID [38, 39] and thereby increasing eligibility for HIV-1 cure interventions.

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

Financial support. This work was supported by the National Institute on Drug Abuse (grant numbers R61-DA047022 and U01-DA036297) and the National Institute of Allergy and Infectious Diseases (grant number K24-AI118591).

Potential conflicts of interest. Aspects of the intact proviral DNA assay are the subject of patent application PCT/US16/28822 filed by Johns Hopkins University with R. F. S. as an inventor and licensed to AcceleVirDx; R. F. S. holds no equity interest in AcceleVirDx. R. F. S. consults for Merck and AbbVie on HIV cure–related issues. K. D. R. is an employee of AcceleVirDx. G. M. L. is an employee of and equity holder in AcceleVirDx. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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