

Paradoxically Greater Persistence of HIV RNA-Positive Cells in Lymphoid Tissue When ART Is Initiated in the Earliest Stage of Infection

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Starting antiretroviral therapy (ART) in Fiebig 1 acute HIV infection limits the size of viral reservoirs in lymphoid tissues, but does not impact time to virus rebound during a treatment interruption. To better understand why the reduced reservoir size did not increase the time to rebound we measured the frequency and location of HIV RNA⁺ cells in lymph nodes from participants in the RV254 acute infection cohort. HIV RNA⁺ cells were detected more frequently and in greater numbers when ART was initiated in Fiebig 1 compared to later Fiebig stages and were localized to the T-cell zone compared to the B-cell follicle with treatment in later Fiebig stages. Variability of virus production in people treated during acute infection suggests that the balance between virus-producing cells and the immune response to clear infected cells rapidly evolves during the earliest stages of infection.

Clinical Trials Registration: NCT02919306.

Keywords. acute HIV infection; antiretroviral therapy; in situ hybridization; HIV reservoir; lymphoid tissues.

The lymphoid tissues are the principal site where human immunodeficiency virus (HIV) replicates and persists before and during antiretroviral therapy (ART), both as a latent infection in resting memory CD4 T cells [1–4] and also in populations of viral RNA-positive (vRNA⁺) cells in which virus production is sustained at low levels [5, 6]. Both of these cellular reservoirs are potential sources of virus to reappear in peripheral blood when ART is interrupted, and thus must be eliminated or substantially reduced in size to achieve either a cure or a sustained remission off ART [7, 8].

One effective strategy to limit the size of the reservoir is to initiate ART early during acute infection [9, 10]. Studies of acute HIV infection have demonstrated that while the reservoir of cells with an integrated provirus is established as early as Fiebig

1 [11], ART given in Fiebig 1, 2, or 3 is associated with a significant decay in this reservoir, but less so if ART is given after Fiebig 3 [12]. Two studies have demonstrated that ART given during acute infection was associated with enhanced functionality of HIV-specific CD4 and CD8 T cells [13, 14], providing a potential mechanism for the rapid decay of this reservoir. However, despite this enhanced immune control and smaller reservoir, recrudescence of viremia with treatment interruption still occurs rapidly even in individuals who start ART in Fiebig 1 [15]. To further characterize the frequency and location of vRNA⁺ cells at the time of acute HIV infection and during follow-up while on ART, we analyzed lymphoid tissues from 76 individuals enrolled in the RV254 acute HIV infection cohort in Bangkok, Thailand (clinicaltrials.gov NCT02919306) [16].

METHODS

Institutional Review Board Approvals

All samples analyzed in this study were obtained with the written consent of participants using protocols approved by institutional review boards/ethical committees at the Walter Reed Army Institute of Research (approval number 1494), Chulalongkorn University, Bangkok, Thailand (approval number 220/51), and the University of Minnesota (approval number 1604M87147). The archived tissues used as historical chronic controls were obtained from participants in an

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institutional review board approved study at the University of Minnesota (approval number 00009301; clinicaltrials.gov NCT04311177).

Plasma HIV RNA

Plasma HIV RNA was measured using either the Roche Amplicor HIV-1 Monitor Test version 1.5 or the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 2.0 (Roche Diagnostics). Lower limits of detection were 50 and 20 copies/mL, respectively.

CD4 T-Cell Counts

CD4⁺ T-cell counts were measured by either single- or dual-platform flow cytometry (Becton-Dickinson Biosciences).

In Situ Hybridization

In situ hybridization (ISH) methods have been previously described [6, 17]. A total of 3 to 5 4- μ m sections separated by 20 μ m were analyzed by RNAscope 2.0. ISH analysis of lymph nodes (LN) obtained from treated individuals was confirmed using the more sensitive RNAscope 2.5 on up to 10 sections/LN as the frequency of vRNA⁺ cell is often very low in this group and we wanted to confirm the results with the more sensitive assay. In the untreated individuals, analysis of 3 to 5 sections was sufficient to accurately characterize the frequency of vRNA⁺ cells. The antisense (for the detection of vRNA) HIV probes used cover approximately 4.5 kb of the genome and were designed to bind to sequences in *gag*, *pol*, *vif*, *vpx* (for SIV), *vpr*, *tat*, *rev*, *vpu* (for HIV) *env*, and *nef*. HIV RNA-specific probes from Advanced Cell Diagnostics were used for HIV RNA ISH (catalog No. 446551 and DNA sense catalog No. 478191; clade A/E). For comparison to chronic infection, we used archival LN tissue from a previous study of individuals with HIV in Minnesota where clade B is the dominant subtype (catalog No. 416111 and DNA sense catalog No. 425531).

Quantitative Image Analysis and Calculation of vRNA⁺ Cells/Million CD4⁺ T Cells

Photographic images were captured and the frequency of vRNA⁺ cells measured and expressed as the total per unit area. These methods have been extensively reviewed [18–20]. Briefly, the area of the tissue was measured using image analysis software and expressed as μm^2 . The tissue was 5 μm thick and so we could calculate the volume of tissue in μm^3 , which we converted to cm^3 . The average density of lymphoid tissue is 1 g/cm^3 [21], providing a measure of cells/g. We also calculated the frequency of positive cells/million CD4⁺ T cells as this provides a means to more directly compare these results to other molecular methods where the denominator is 1 million CD4 T cells. To make this conversion, we first excluded the medullary cords from the captured images of LN stained with antibodies against CD4 and determined the percent area of the tissue that contained CD4⁺ T cells using image

analysis methods we had previously developed [22–24]. We then measured the absolute frequency of nucleated cells in the tissue sections using Image J. From these 2 measures we determined the absolute frequency of CD4⁺ T cells in the tissue section (nucleated cells \times % area CD4⁺ cells). We then expressed the frequency of vRNA⁺ cells per absolute number of CD4⁺ T cells and determined what the number of vRNA⁺ cells would be per 10⁶ CD4⁺ T cells. To validate this approach, we hand counted the absolute number of CD4⁺ T cells in 5 sections that had been stained with antibodies to CD4, from that determined the number of vRNA⁺ cells per million CD4⁺ T cells, compared these results to the method described above, and found excellent agreement between the 2 methods ($r^2 = .99$, $P = .0002$; Supplementary Figure 1).

Statistical Analysis

Continuous values were summarized using medians, quartiles, and ranges. Categorical variables were summarized using proportions. Frequency of HIV vRNA⁺ cells/g and vRNA⁺ cells/million CD4 T cells in the LN were log base 10 transformed prior to statistical analysis. Zero values recorded for vRNA⁺ cells/g or vRNA⁺ cells/million CD4 T cells were imputed at the minimum detectable value of 1 cell. Due to small sample sizes, Fiebig stages 4 and 5 were grouped together for the analyses described below, unless otherwise noted. Unadjusted P values and Holm adjusted P values are presented for each regression analysis.

Separate analyses were conducted for comparing vRNA⁺ frequency at baseline and during ART. A multivariable linear regression model was used to compare differences in log base 10 transformed vRNA⁺ cells/g at baseline (measured by RNAscope 2.0) between the 4 distinguished Fiebig groups while adjusting for age at biopsy. For the analysis of biopsies taken during ART, a logistic generalized estimating equation (GEE) model, with independence working correlation, was used to compare differences in the proportion of vRNA⁺ biopsies (measured by RNAscope 2.0) between the 4 distinguished Fiebig groups after adjusting for time on ART and age at biopsy. A multivariable linear GEE regression model with independence working correlation was used to compare log base 10 transformed vRNA⁺ cells/g (measured by RNAscope 2.5) during ART between Fiebig groups (Fiebig 1 vs >1), after adjusting for time on ART and age at biopsy. A similarly constructed model was used for comparing log base 10 transformed vRNA⁺ cells/million CD4 T cells between Fiebig groups. Results from models unadjusted for other covariates (age at biopsy and time on ART) are also presented as a sensitivity analysis.

A linear regression model was used to evaluate the relationship between pretherapy log base 10 transformed plasma viral load and log transformed vRNA⁺ frequency (measured by RNAscope 2.0) at baseline and a logistic GEE model was used to evaluate the relationship pretherapy log base 10 transformed

plasma viral load and detection of vRNA⁺ cells (measured by RNAscope 2.0) after therapy was begun.

For comparing the anatomical location of vRNA⁺ cells detected during ART, Fiebig 1 and 2 and Fiebig 3–5 were grouped together because of the low frequency of samples. A multivariable binomial model with over-dispersion parameter was fit to compare the association between detecting vRNA⁺ cells in B-cell follicles (as opposed to the T-cell zone) and Fiebig stage while adjusting for age at sampling. Results from the model unadjusted for other covariates are also presented.

RESULTS

The cohort consisted of 76 individuals (2 females and 74 males) participating in the RV254 trial in Bangkok, Thailand (Table 1). The median age at entry was 27 years (range, 18–65 years). Participants were divided into 2 groups; the first group was composed of 43 individuals who were immediately biopsied at the time of diagnosis of acute infection (median 2 days after diagnosis; quartiles 0, 4 days). The second group was composed of 33 individuals who deferred biopsy until after a median of 51 (quartiles 48, 145) weeks of ART. The specific clade of infecting virus was determined for 59 of the participants and, of those, 52 were AE, 2 were nontypeable, and 5 were AE/B recombinants. Of the 43 that were immediately biopsied at the time of diagnosis, 4 individuals (1 each diagnosed in Fiebig 1, 3, 4, and 5) were biopsied a second time after a median of 48 (quartiles 47, 48) weeks of ART. Of the 33 individuals that deferred biopsy until after a median of 51 weeks of ART, 3 individuals had 2 biopsies; 1 individual was identified in Fiebig 2 and 2 were identified in Fiebig 3. In 1 of the Fiebig 3 participants the first sample was obtained 26 weeks after starting ART and the second at 97 weeks. In the other 2 participants the first sample was obtained between 143 and 144 weeks after the start of ART and the second between 240 and 242 weeks.

Participants in both groups were stratified into 5 groups based on Fiebig stage 1–5 of HIV infection at time of diagnosis and ART initiation [25] (Table 1). As expected, there was a rapid exponential increase in plasma HIV RNA depending on Fiebig stage at the time of diagnosis from a median of 11 969 (quartiles 7165, 50 217) HIV RNA copies/mL in Fiebig stage 1 to a peak median viral load in Fiebig stage 3 of 2 651 859 (quartiles 820 668, 11 928 175) copies/mL (Supplementary Figure 2). The median CD4 T-cell count was 575 (quartiles 456, 637) cells/ μ L in Fiebig stage 1, 293 (quartiles 186, 338) cells/ μ L in Fiebig stage 2, 314 (quartiles 234, 385) cells/ μ L in Fiebig stage 3, 438 (quartiles 234, 473) cells/ μ L in Fiebig stage 4, and 298 (quartiles 271, 490) cells/ μ L in Fiebig stage 5 (Table 1).

We analyzed the frequency of vRNA⁺ cells in the LN from the individuals before ART was begun by RNAscope 2.0 ISH. In Fiebig 1, the median frequency of vRNA⁺ cell was 3.5×10^4 (quartiles: 3.7×10^3 , 1.1×10^5) vRNA⁺ cells/g LN (Figure 1A).

To put this into perspective, this is the equivalent of 12 vRNA⁺ cells/ 10^6 CD4 T cells (Figure 1B and Supplementary Table 1). This pool of vRNA⁺ cells rapidly expanded to approximately 7-fold higher median frequencies in individuals in Fiebig 2 and Fiebig 3 (adjusted *P* values = .0265 and .0140, respectively; Supplementary Table 2). In addition, we found the frequency of vRNA⁺ cells in LN was significantly associated with the pretherapy plasma viral load (*P* < .001; Figure 1C). However, there was no association between pretherapy plasma viral load and detection of vRNA⁺ cells after therapy began (*P* = .26).

In the analysis of lymphoid tissues from the treated group using RNAscope 2.0 we found that 19/40 (48%) biopsies had vRNA⁺ cells detected at a median frequency of 5.3×10^4 cells/g (quartiles: 2.7×10^4 , 1.3×10^5 cells/g). However, of the 31 biopsies collected from 28 individuals starting ART in Fiebig stages 2–5, 11 biopsies (35%) had vRNA⁺ cells detected after 25–243 weeks of ART and individuals who initiated ART during Fiebig 4–5 had significantly reduced odds of having a vRNA⁺ detected in LN tissues compared to individuals who started ART during Fiebig 1 (Supplementary Table 3). These data suggest that starting ART in Fiebig 1 was less effective at suppressing virus production in the LN than starting ART in the later stages of acute infection. To confirm this finding, we repeated the ISH analysis in all of the ART-treated group using the more sensitive RNAscope 2.5 on up to 10 sections/LN and detected vRNA⁺ cells in 28/40 (70%) of LN biopsies at a median frequency of 4.7×10^4 vRNA⁺ cells/g (quartiles: 2.7×10^4 , 8.5×10^4 cells/g). vRNA⁺ cells were detected in 8/9 (88.9%) biopsies from individuals starting ART in Fiebig 1, 4/8 (50%) biopsies in Fiebig 2, 10/14 (70%) in Fiebig 3, and 6/9 (67%) in Fiebig 4–5. In Fiebig 1, the median frequency of detection was 5.9×10^4 cells/g (quartiles: 3.5×10^4 , 1.6×10^5 cells/g), close to those measured before treatment; however in Fiebig 2–5 the median frequency was significantly less at 1.7×10^4 cells/g (quartiles: 0, 5.33×10^4 cells/g) (*P* = .03; Figure 2 and Supplementary Table 4). We show in Figure 3 representative images from a LN obtained in Fiebig 1 prior to ART (Figure 3A) and a LN obtained after ART (Figure 3B). We also show representative sections from a LN obtained before ART in Fiebig 3 (Figure 3C) and another participant diagnosed in Fiebig 3 from a LN biopsy obtained during ART (Figure 3D). Thus, initiation of ART in the earliest stage of infection paradoxically had less impact on reducing the frequency of vRNA⁺ cells in LNs compared to initiating treatment at later stages.

We also determined the anatomic location of vRNA⁺ cells during ART as B-cell follicles are an independent reservoir for HIV-infected cells because CD8 T cells are relatively restricted from follicles due to lack of CXCR5 expression [26]. If CD8 T cells were not able to clear HIV-infected cells when ART is started too early, we might expect to find significant differences between the frequency of infected cells in B-cell follicles and the parafollicular T-cell zone. We manually counted the frequency

Table 1. Demographic Characteristics of the Cohort

Characteristic	Fiebig 1		Fiebig 2		Fiebig 3		Fiebig 4		Fiebig 5	
	Immediate ^a	Deferred	Immediate	Deferred	Immediate	Deferred	Immediate	Deferred	Immediate	Deferred
No. of individuals	7	8	8	7	19	11	5	4	4	3
Age at first biopsy, y	24.0 (22.5, 25.5)	27.5 (26.8, 32.0)	22.5 (21.5, 27.5)	26.0 (22.5, 32.5)	28.0 (23.0, 33.5)	30.0 (23.0, 35.0)	27.0 (23.0, 34.0)	26.0 (24.0, 28.2)	32.0 (23.8, 39.2)	29.0 (27.0, 31.5)
Male, No. (%)	7 (100)	7 (87.5)	8 (100)	7 (100)	19 (100)	10 (90.9)	5 (100)	4 (100)	4 (100)	3 (100)
Duration of ART at first biopsy, d	2.0 (0.5, 3.0)	516.5 (349.8, 774.0)	0.5 (0.0, 2.5)	337.0 (251.5, 345.0)	2.0 (0.0, 4.5)	349.0 (275.0, 1081.0)	2.0 (2.0, 3.0)	516.5 (299.8, 773.2)	1.0 (0.8, 2.5)	687.0 (512.0, 689.5)
CD4 at diagnosis	575 (414, 616)	570 (456, 708)	256 (198, 314)	338 (186, 396)	321 (236, 420)	308 (240, 359)	473 (456, 519)	234 (221, 278)	336 (282, 437)	276 (271, 440)
CD4 at first biopsy	575 (414, 616)	842 (653, 1004)	256 (198, 314)	609 (485, 706)	321 (236, 420)	579 (514, 700)	473 (456, 519)	450 (370, 535)	336 (282, 437)	382 (362, 1006)
Plasma viral load at diagnosis, log ₁₀ transformed	4.1 (3.8, 4.4)	4.4 (3.9, 4.8)	5.8 (5.3, 6.2)	5.6 (5.4, 6.8)	6.2 (5.7, 6.6)	7.1 (6.3, 7.2)	5.5 (5.2, 6.8)	6.5 (6.0, 6.9)	5.9 (5.7, 6.2)	5.5 (5.2, 6.3)
Plasma viral load at first biopsy, log ₁₀ transformed	4.1 (3.8, 4.4)	<1.3 (<1.3, <1.3)	5.8 (5.3, 6.2)	<1.3 (<1.3, <1.3)	6.2 (5.7, 6.6)	<1.3 (<1.3, <1.3)	5.5 (5.2, 6.8)	<1.3 (<1.3, <1.3)	5.9 (5.7, 6.2)	<1.3 (<1.3, <1.3)

Data are median (quartiles) unless otherwise specified.

Abbreviation: ART, antiretroviral therapy.

^aTime of first biopsy: immediate or deferred.

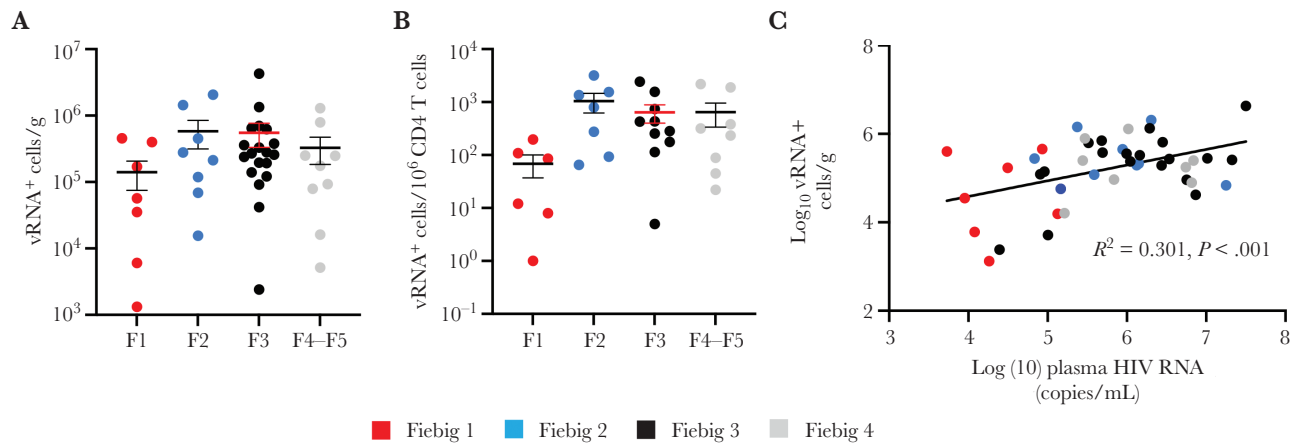


Figure 1. Measures of HIV in lymph nodes of the untreated group. HIV RNA in situ hybridization and quantitative image analysis were used to measure the frequency of HIV vRNA⁺ cells/g lymphoid tissue in those biopsied immediately at the time of diagnosis (A) expressed as vRNA⁺ cells/g and (B) as vRNA⁺ cells/million CD4 T cells. C, The relationship between log frequency of vRNA⁺ cells/g and the log plasma viral load is significant ($R^2 = 0.301$, $P < .001$, linear regression). Abbreviations: F, Fiebig; vRNA⁺, virus RNA positive.

of vRNA⁺ cells in the T-cell zone and in the B-cell follicles in a subset of 27 people in the ART group with detectable vRNA⁺ cells in LN (8 in Fiebig 1, 4 in Fiebig 2, 10 in Fiebig 3, and 5 in Fiebig 4–5). For comparison, we analyzed 10 archived LN samples from a previous study of people living with HIV in Minnesota who started ART during chronic infection and had been on ART for at least 1 year. The sample set consisted of 10 individuals from a study completed at the University of Minnesota and were collected and processed using the same protocols. All were men, the mean age was 43.6 years (range, 20–68 years) and the mean CD4 T-cell count at the time of biopsy was 535 cells/ μ L (range, 375–728 cells/ μ L). We pooled data from Fiebig

1 and 2 ($n = 12$), and Fiebig 3–5 ($n = 15$) because of the low frequency of detectable vRNA⁺ cells. We found that in those individuals treated in Fiebig 1–2, the median percentage of vRNA⁺ cells that were in the B-cell follicle was 16.67% (quartiles: 5.60%, 23.30%). However, in those starting therapy in Fiebig 3–5 it was 33.33% (quartiles: 11.85%, 54.42%) and in chronic infection it was 41.17% (quartiles: 37.50%, 48.37%). Individuals treated in Fiebig 1–2 had significantly fewer vRNA⁺ cells in the B-cell follicle and more in the T-cell zone compared to individuals starting therapy during chronic infection (adjusted P value = .01; [Figure 4](#) and [Supplementary Table 5](#)). When ART is begun during the earliest stages of infection, 84.6% of detected

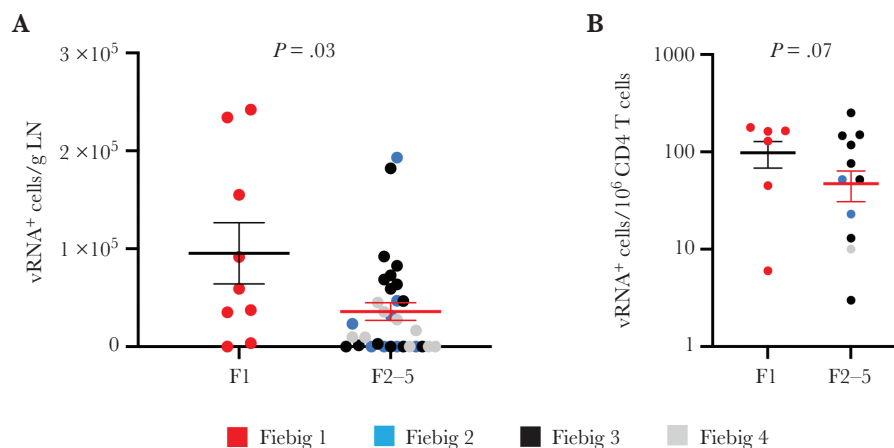


Figure 2. The frequency of vRNA⁺ cell was significantly higher in people treated during Fiebig 1 than other Fiebig stages. A, The frequency of vRNA⁺ cells/g LN tissue in Fiebig 1 compared to the frequency from LN in participants from Fiebig stages 2–5 ($P = .03$). A multivariable linear generalized estimating equation regression model with independent working correlation was used to compare log base 10 transformed vRNA⁺ cells/g (measured by RNAscope 2.5) during ART between Fiebig groups (Fiebig 1 vs >1), after adjusting for time on ART and age at biopsy ([Supplementary Table 5](#)). B, The frequency of cells/g was converted to frequency per million CD4 T cells in the LN ($P = .04$) and a similarly constructed model was used for comparing log base 10 transformed vRNA⁺ cells/million CD4 T cells between Fiebig groups. Results from models unadjusted for other covariates (age at biopsy and time on ART) are also presented as a sensitivity analysis ([Supplementary Table 5](#)). Abbreviations: ART, antiretroviral therapy; F, Fiebig; LN, lymph node; vRNA⁺, virus RNA positive.

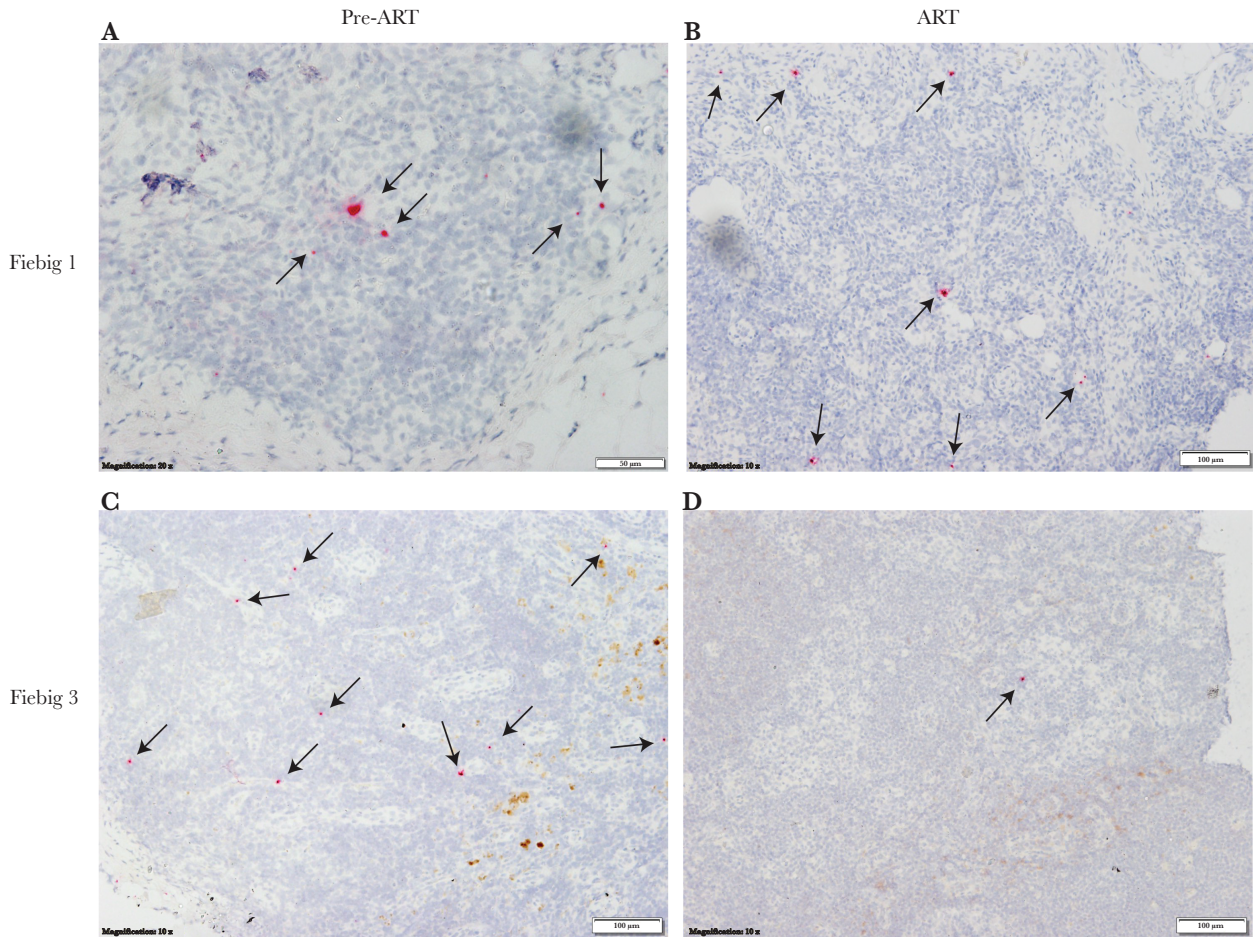


Figure 3. Representative images of lymph nodes from Fiebig 1 at the time of diagnosis (A) and after ART (B) and from Fiebig 3 at diagnosis (C) and after ART (D). A, Scale bar 50 μm . B, C, and D, scale bar 100 μm . Arrows show vRNA⁺ cells. Abbreviations: ART, antiretroviral therapy; vRNA⁺, virus RNA positive.

vRNA⁺ cells were in the T-cell zone, supporting a model where HIV-specific CD8 T cells are unable to clear these cells.

DISCUSSION

This study yielded 3 important discoveries about viral dynamics in acute infection, especially when ART is initiated in the earliest stages of the infection. First, most individuals who start ART during acute infection have evidence for ongoing virus production in LN. Second, these cells were significantly more frequent if ART was started in Fiebig 1. Third, 84.6% of vRNA⁺ cells were detected in the T-cell zone if ART was started in Fiebig 1 or 2 compared to later stages of infection, where it was closer to 50%.

We undertook these studies because we were interested to understand the dynamic nature of viral reservoirs as they are established in lymphatic tissues during acute HIV infection, and the impact of early ART on these reservoirs. We found a rapid increase in vRNA⁺ cells during the earliest stages of infection such that by Fiebig 2 the frequency of vRNA⁺ cells was the same as when ART was started in later Fiebig stages. In our initial analysis of LNs from ART-treated individuals we found that

48% of biopsies continued to have detectable vRNA⁺ cells and we found an unexpectedly high frequency of these cells when ART was started in Fiebig 1 compared to later stages of acute infection. To confirm this finding, we used the recently developed and more sensitive RNAscope 2.5 ISH procedure and found that 70% of all biopsies had detectable vRNA⁺ cells despite full suppression for an average of 91 weeks. This finding, by itself, is surprising because these individuals were fully suppressed in peripheral blood and yet we have evidence for ongoing virus production in the majority of the participants.

There are several possible explanations for this. We, and others, have found that concentrations of antiretroviral drugs (ARVs) in lymphoid tissues are significantly lower and subtherapeutic compared to what is measured in simultaneously collected peripheral blood mononuclear cells [5–7]. This is true in acute infection as well, as we recently demonstrated in the RV254 cohort [27]. We have shown an indirect and significant correlation between the frequency of vRNA⁺ cell in lymphatic tissues with the intracellular concentration of the active moiety of the drug [5]. The frequency of vRNA⁺ cell detection increases as drug concentration decreases, suggesting

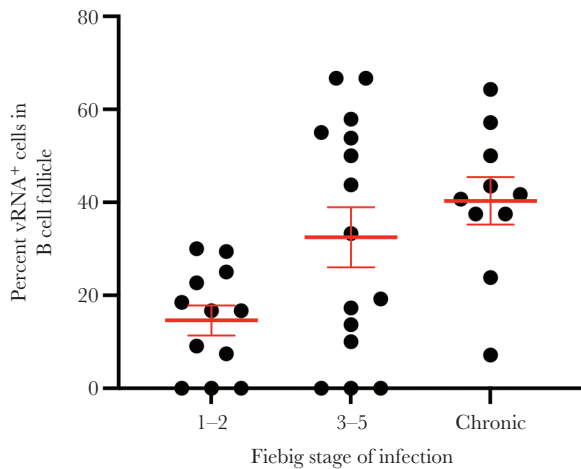


Figure 4. The percent of vRNA⁺ cells found in B-cell follicles during ART at Fiebig 1–2, Fiebig 3–5, and in a group of HIV-infected people who started ART in chronic HIV infection (data generated from archived tissues). Adjusted *P* value = .01 for comparing Fiebig 1–2 to chronic infection. Abbreviations: ART, antiretroviral therapy; vRNA⁺, virus RNA positive.

ART could suppress virus production if the concentration was high enough. A recent study by Scholz et al demonstrated, using mass spectrometry to directly visualize drug concentration in LN tissues, that 31% of LN tissue and 25% of vRNA⁺ cells were not exposed to any ARV drugs [28]. The consistent finding of reduced and subtherapeutic concentration of ARV drugs in the LN tissues, where most virus replication occurs, offers a potential explanation for why most of the treated participants in this study had evidence for ongoing virus production.

The second important finding of these studies was that the frequency of vRNA⁺ cells in ART-treated individuals was significantly higher in people who began therapy in Fiebig 1 as opposed to Fiebig stages 2–5. It is possible, even likely, that a potential mechanism to explain this finding is that HIV-specific T-cell responses are somehow limited if ART is begun in the earliest stages of HIV infection. Ndhlovu et al showed that HIV-specific CD8⁺ T cells displayed a hypermetabolic phenotype during Fiebig 1 and 2, promoting survival, but were functionally impaired [13] and Trautman et al showed that this impaired CD8 T-cell phenotype persisted into Fiebig 5 infection, when the individual enters the chronic phase of HIV infection [14]. Takata et al showed that individuals in RV254 who initiated ART in the earliest Fiebig stages had delayed expansion of CD8 T cells compared to individuals who initiated ART after Fiebig stage 3, when HIV-specific CD8 T cells expanded rapidly and were associated with a steeper viral load decrease after starting ART [29]. Thus, paradoxically, while initiating early ART may lower viral reservoirs by inhibiting virus replication early in the infection and preserve other immune functions, a diminished ability of CD8⁺ cells to target and eliminate HIV-infected LN cells may explain our finding of higher long-term virus production in LN from individuals starting ART in Fiebig 1.

Finally, we found that among those individuals starting ART in Fiebig 1 and 2, 84.6% of vRNA⁺ cells were located in the T-cell zone and only 15.4% in the B-cell follicle. If therapy is begun in the later stages of acute infection or during chronic infection, up to 50% of vRNA⁺ cells are in the B-cell follicles. This is because B-cell follicles are a distinct anatomic reservoir for HIV-infected cells as CD8⁺ T cells are restricted from entering follicles due to lack of expression of CXCR5 [26, 30] and therefore lack an important mechanism for clearance of infected cells. It is possible that more vRNA⁺ cells persist in the T-cell zone in Fiebig 1 due to the inability to clear virally infected cells by CD8⁺ T cells with relatively decreased function. We do not know if there are differences in the mechanism of virus reactivation during treatment interruption, but this could be the source of virus rebound from individuals treated in Fiebig 1 whereas the source from individuals starting in later Fiebig stages may be the B-cell follicle.

These data provide further insight into the nature of the dynamic reservoir that is established very early in HIV infection and the complex interplay between the immune response and kinetics of virus replication as ART is begun during acute HIV infection. Further studies are needed to determine the mechanism for persistent virus production in LNs when treatment is begun in the earliest stages of infection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Disclaimer. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The views expressed are those of the authors and should not be construed to represent the positions of the US Army or the Department of Defense (DoD).

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form

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