Immunologic and virologic parameters associated with HIV DNA reservoir size in people living with HIV receiving antiretroviral therapy

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Background. A better understanding of the dynamics of HIV reservoirs in CD4+ T cells of people with HIV (PWH) receiving antiretroviral therapy (ART) is crucial for developing therapies to eradicate the virus.

Methods. We conducted a study involving 28 aviremic PWH receiving ART with high and low levels of HIV DNA. We analyzed immunologic and virologic parameters and their association with the HIV reservoir size.

Results. The frequency of CD4+ T cells carrying HIV DNA was associated with higher pre-ART plasma viremia, lower pre-ART CD4+ T cell counts, and lower pre-ART CD4/CD8 ratios. During ART, the High group maintained elevated levels of intact HIV proviral DNA, cell-associated HIV RNA, and inducible virion-associated HIV RNA. HIV sequence analysis showed no evidence for preferential accumulation of defective proviruses nor higher frequencies of clonal expansion in the High versus Low group. Phenotypic and functional T-cell analyses did

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not show enhanced immune-mediated virologic control in the Low versus High group. Of considerable interest, pre-ART innate immunity was significantly higher in the Low versus High group.

Conclusions. Our data suggest that innate immunity at the time of ART initiation may play an important role in modulating the dynamics and persistence of viral reservoirs in PWH.

Keywords. HIV reservoirs; antiretroviral therapy; plasma viremia; CD4+ T cell count; immune cells

INTRODUCTION

The development of antiretroviral therapy (ART) has led to remarkable improvements in the health and life expectancy of people with HIV (PWH) [1]. However, ART is not curative, in part due to the persistence of HIV reservoirs [2-4]. These infected cells likely account for the rapid plasma viral rebound in PWH following analytical treatment interruption (ATI) [5] and therefore are considered a major impediment to HIV eradication. Yet, despite more than two decades of intense research, the precise mechanism(s) contributing to the establishment and/or maintenance of HIV reservoirs has not been fully delineated, and numerous attempts aimed at eliminating infected cells have not been widely successful thus far [6-8].

The HIV reservoir is established early during the primary infection [9]. Shortly after the initiation of ART, the HIV reservoir decays in multiple phases in PWH, first rapidly, then followed by a slower decay [10-12]. The size of the viral reservoir remains unperturbed in the majority of PWH despite years to decades of ART, in part due to the stability of the HIV provirus in the CD4+ T cells. Previous studies have demonstrated that certain immunologic and virologic parameters, namely pre-ART plasma viremia, CD4+ T cell counts, and/or CD4/CD8 T cell ratios, can affect the HIV DNA burden in PWH during ART [13-17]. Other factors, including time to suppress plasma viremia following ART initiation, the number of viral blips during ART [16], the timing of ART initiation [18], the levels of HIV-specific T-cell immunity [19, 20] and T-cell activation and exhaustion [21, 22] have also been associated with the size of HIV reservoirs in PWH receiving ART. However, studies addressing the intact versus defective state of HIV proviral DNA in the CD4+ T cells prior to and following ART as well as the role of pre-ART innate immunity in modulating the post-ART HIV reservoir size in aviremic PWH receiving antiretroviral drugs for extended periods, are lacking. We conducted the present study to address these issues.

METHODS

Please see the Supplemental Methods for detailed experimental procedures.

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Study participants

Twenty-eight PWH enrolled in long-term longitudinal HIV cohort study (Clinicaltrials.gov ID NCT00039689) who had been receiving suppressive ART for a median of 8 years were selected based on their HIV DNA burden (Table 1). PWH with total HIV DNA <600 and >2,000 copies per 10^6 CD4+ T cells were selected for stratification into two groups. All study participants were infected with HIV subtype B. Leukapheresis products were obtained from the study participants in accordance with clinical protocols approved by the Institutional Review Board of the National Institutes of Health. All study participants provided informed consent.

RESULTS

Characteristics of study participants.

A total of 28 PWH were studied longitudinally prior to (pre-ART) and following (post-ART) the initiation of clinically effective ART for a median of 8.0 years (range 2.3-15.3; Table 1). The study participants were divided into two groups, “High” and “Low”, based on the copy number of total HIV DNA per 10^6 CD4+ T cells at the post-ART time point. The High (n=13) and Low (n=15) groups had a median of 2,927 (range 2,238-4,910) and 359 (range 169-594) copies of HIV DNA per 10^6 CD4+ T cells, respectively (Figure 1A). There were no significant differences in age and duration of ART between the groups (Table 1). The CD4+ T cell counts were comparable (P=0.072) between the two groups at the post-ART time point; however, the High group had higher CD8+ T cell counts and lower CD4/CD8 ratios than the Low group (Table 1). In addition, the High group had significantly lower CD4+ T cell counts and CD4/CD8 ratios and higher plasma viremia compared to the Low group at the pre-ART time point (Table 1). Of note, while there was a significant increase in the CD4+ T cell counts and CD4/CD8 ratios in both groups post-ART, only the Low group experienced a significant decrease in CD8+ T cell counts (Supplementary Figure 1A), suggesting viral persistence and/or residual immunologic abnormalities in the High group.

Comparison of virologic parameters between the High and Low groups.

To evaluate the size of HIV reservoirs in the CD4+ T cell compartment of the study participants prior to and following the initiation of ART, we conducted several molecular- and cellular-based assays. As shown in Figure 1A, the levels of post-ART cell-associated HIV RNA (P<0.0001) and inducible virion-associated HIV RNA (P<0.0001) were significantly higher in the High compared to the Low group. We then performed the intact proviral DNA assay (IPDA) to compare the composition of HIV DNA in the CD4+ T cells of both groups before and after ART initiation. The levels of pre- and post-ART intact (P=0.0006 and P<0.0001), 5’defective (P=0.0001 and P<0.0001), and 3’defective (P<0.0001 and P<0.0001) HIV DNA were significantly higher in the High group compared to those of the Low group (Figure 1B). All three
forms of proviral DNA decreased significantly following the initiation of ART in both groups (Figure 1B). Of note, the ratios between intact and defective proviral DNA were comparable between the groups, suggesting that the higher total HIV DNA burden seen in the High group was not driven by the accumulation of defective viral DNA over time (Supplementary Figure 1B). Previous studies have shown that several pre-ART parameters are predictive of the total HIV DNA burden in the CD4\(^+\) T cells of PWH receiving ART [13, 15, 17, 23]; however, the impact of such variables on the levels of intact and defective HIV proviral DNA and inducible virus has not been examined thus far. To this end, we explored correlations between the pre-ART CD4\(^+\) T cell counts and plasma viremia and the frequency of CD4\(^+\) T cells carrying intact and defective HIV proviral DNA at the post-ART study point. The pre-ART CD4\(^+\) T cell counts negatively correlated with the levels of intact \((P=0.0360)\), 5’(\(P=0.0001\)), and 3’(\(P<0.0001\)) defective HIV proviral DNA (Figure 1C). In addition, the pre-ART plasma viremia positively correlated with the levels of intact \((P=0.0011)\), 5’(\(P=0.0120\)), and 3’(\(P=0.0107\)) defective HIV proviral DNA (Figure 1C). Unlike pre-ART CD4\(^+\) T cell count, the pre-ART plasma viremia correlated most significantly with the post-ART intact proviral DNA. We then evaluated the relationship between the above pre-ART parameters and the levels of inducible virion-associated HIV RNA in the CD4\(^+\) T cells of the study participants during ART. There were statistically significant correlations between both pre-ART CD4\(^+\) T cell counts \((P=0.0009)\) and pre-ART plasma viremia \((P=0.0083)\) and the level of inducible virion-associated HIV RNA at the post-ART time point (Figure 1C). Taken together, our data suggest that pre-ART immunologic and virologic parameters have distinct effects on the composition and replication competency of HIV viral reservoirs in PWH during suppressive ART.

**Dynamics and composition of HIV DNA reservoirs.**

We calculated the half-lives of intact, 5’defective, and 3’defective HIV proviral DNA in the CD4\(^+\) T cells of the study groups to investigate the effect of a differential decay rate of viral DNA on the maintenance of HIV reservoirs during ART (0.5-8 years following initiation of ART). As shown in Figure 2A and Supplementary Figure 2, the decay rate of intact proviral DNA was significantly faster (median 3.76 years, range 1.48-11.1) than that of 5’(median 13.45, range 3.24-80) and 3’(median 11.05, range 3.14-80) defective DNA \((P<0.0001)\) in both study groups, corroborating previous findings [11, 12, 24]. Of note, we found no significant difference in the decay rate of intact or defective HIV proviral DNA between the study groups (Figure 2A).

Next, we assessed the distribution of post-ART cells carrying intact HIV proviral DNA among different T-cell subsets of the study participants. Except for effector CD4\(^+\) T cells, all other subsets (naïve, central memory, and transitional memory) in the High group had elevated levels of intact HIV DNA compared to counterparts in the Low group (Figure 2B). However, after adjusting for the percentage of each subset within the total CD4\(^+\) T cell population (Figure 2C), the contribution of each CD4\(^+\) T cell subset to the pool of infected cells carrying intact HIV DNA was not significantly different between the two groups (Figure 2D). We then investigated sequence diversity and clonality of the persistent HIV reservoirs between the two groups during
ART. As shown in Figure 2E, the degree of sequence diversity in the HIV envelope (V1-V5) in total CD4+ T cells was not significantly different between the two groups. In addition, the degree of clonality of HIV env in total and subsets of CD4+ T cells was comparable between the groups (Figures 2E and F). Taken together, our data suggest that the elevated HIV DNA burden observed in the High group is unlikely due to a slower decay, a higher diversity of HIV env sequences, or accelerated clonal expansion of infected CD4+ T cells carrying HIV DNA.

T cell immunity and HIV DNA reservoir size.

To better understand the role of the host immune response in the persistence of HIV reservoirs, we conducted phenotypic and functional analyses of T cells of the study participants. First, we examined the levels of exhaustion (TIGIT and PD-1) and activation (CD38/HLA-DR) markers on the CD4+ and CD8+ T cells (Figure 3A). The levels of pre-ART TIGIT and PD-1 on the CD4+ T cells and CD38+HLA-DR+ on both CD4+ and CD8+ T cells were significantly elevated in the High compared to the Low group. Of note, the expression of TIGIT and PD-1 on CD4+ T cells decreased in the High, but not in the Low group following initiation of ART, yet it remained significantly elevated in the High compared to the Low group at the post-ART time point. In contrast, the levels of CD38+HLA-DR+ on CD4+ and CD8+ T cells significantly decreased following the initiation of ART in both groups and did not differ post-ART. We then performed a 21-parameter phenotypic analysis of peripheral blood T cells at both pre- and post-ART time points using spectral flow cytometry and Uniform Manifold Approximation and Projection (UMAP; Figure 3B). From the 15 meta-clusters generated by the FlowSOM algorithm based on the expression of 19 surface markers, four clusters significantly differed between the two groups at pre- and/or post-ART time points (Figures 3C-D). Cluster 2, memory CD4+ T cells expressing co-stimulatory molecule CD28, was significantly enriched in the Low group compared to the High group pre-ART and increased in both groups post-ART. The Low group also had a significantly higher percentage of naïve CD4+ T cells (Cluster 8) at both pre- and post-ART time points. In contrast, cluster 4, comprised of transitional memory CD8+ T cells expressing markers of immune activation (CD38 and HLA-DR) and exhaustion (TIGIT, PD-1, and 2B4), was significantly enriched in the High compared to the Low group pre-ART and then significantly decreased in both groups post-ART. Cluster 9, comprised of effector memory and terminally differentiated CD8+ T cells expressing CD226 and 2B4, decreased significantly in both groups following initiation of ART, although it remained significantly enriched in the High versus the Low group at the post-ART time point (Figure 3D).

To gain further insight into the potential role of T cell immunity in modulating HIV reservoir size, we examined HIV-specific CD4+ and CD8+ T cells in the study participants by performing intracellular cytokine staining following stimulation with a pool of peptides spanning the entire HIV Gag protein. As shown in Figure 3E, the frequencies of polyfunctional HIV Gag-specific CD4+ (IFN-γ+TNF-α+IL-2+CD40L+) or CD8+ (IFN-γ+TNF-α+MIP-1β+) T cells at pre- or post-ART time points were not significantly different between the two groups, suggesting that HIV Gag-specific cytolytic T cells unlikely contributed significantly to the modulation of the HIV DNA size.
DNA reservoir size. We then measured the levels of the cytokine IL-6, chemokines MIP-1α and RANTES, cytotoxic proteins Granzyme B and Perforin, and markers of immune activation IL-2Rα, PD-L1, IP-10 in the plasma of study participants at the pre- and post-ART time points. The levels of Granzyme B, PD-L1, and IP-10 were significantly higher in the High group compared to the Low group at the pre-ART time point; however, no differences were observed at the post-ART time point (Supplementary Figure 3). These data suggest that higher levels of immune activation in the High compared to the Low group prior to ART initiation could potentially contribute to the shaping of the HIV reservoir despite normalization following ART.

Impact of pre-ART innate immune response on the persistence of HIV reservoirs.

Innate immunity plays an important role in the early control of HIV infection [25, 26] and, therefore, may contribute to the modulation of the viral reservoir size in PWH. To address this issue, we conducted phenotypic and functional characterization of plasmacytoid dendritic cells (pDCs) and natural killer (NK) cells of the study participants prior to and following the initiation of ART. The percentages of pDCs in the peripheral blood at pre- and post-ART time points were not significantly different between the two groups (Figure 4A). However, upon stimulation with CpG oligonucleotides, the pDCs of the Low group produced significantly higher levels of IFN-α2 than those of the High group at the pre-ART time point (P=0.0074, Figure 4A). Next, we conducted phenotypic analyses of NK cells to evaluate whether there were differences in cytotoxic potential between the two groups. We found a significantly higher frequency of CD56dimCD16+ NK cells, a mature NK cell subset with potent cytolytic activity [27], in the Low compared to the High group at the pre-ART time point (P=0.0234, Figure 4B). Similarly, we found higher expression of CD57, a marker of highly mature CD56dimCD16+ NK cells with a more potent CD16-induced cytotoxicity [28, 29] in the Low compared to the High group at the pre-ART time point (P=0.0045, Figure 4B). These differences between the study groups were not observed at the post-ART time points (Figures 4A and B). Taken together, these data suggest a potential role of innate immunity in restricting and shaping the size of HIV reservoirs prior to and following the initiation of ART.

Correlation between immunologic and virologic markers and the size of HIV reservoirs.

Finally, we used a correlation matrix to summarize the relationship between the HIV DNA burden at the post-ART time point and all immunologic and virologic variables examined at pre- and post-ART time points in this study. Each row and column in the matrix represent an individual parameter, ordered by the strength of the correlation with the post-ART HIV DNA (Figure 5) or by hierarchical clustering (Supplementary Figure 4). The parameters that positively correlated with the size of the HIV DNA reservoir at the time of study included pre- and post-ART intact and defective HIV DNA, cell-associated HIV RNA, inducible virion-associated HIV RNA, pre-ART plasma viremia, the frequencies of CD4+ and CD8+ T cells with a transitional/effecter memory phenotype expressing activation and exhaustion markers, and pre-ART IP-10, PD-L1 and Granzyme B levels in plasma (Figure 5). The parameters that negatively

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correlated with the size of HIV DNA reservoirs included pre-ART CD4+ T cell count, naïve T-cell phenotype, CD4/CD8 ratios, the capacity of pre-ART pDCs to produce IFN-α2, and the frequency of pre-ART NK cells with greater cytotoxic potential.

DISCUSSION

In the present study, we examined the two groups of PWH receiving ART that differed by their HIV proviral DNA burden in the CD4+ T cell compartment. We conducted comprehensive analyses of immunologic and virologic parameters that could explain what set these two study groups apart. We demonstrated that the PWH with higher pre-ART plasma viremia, lower pre-ART CD4+ T cell counts, and lower pre-ART CD4/CD8 ratios may allow the establishment of a larger size of CD4+ T cell reservoirs harboring total HIV DNA as well as intact proviral DNA, cell-associated HIV RNA, and inducible virion-associated viral RNA. Despite the significant differences in the size of HIV reservoirs between the two groups during ART, the similarities observed in the ratio of intact-to-defective HIV DNA and HIV env sequences argued against the preferential accumulation of defective proviruses or a higher frequency of clonal expansion as the reason for the higher HIV burden seen in the High versus Low group. Longitudinal phenotypic analyses of T cells of the study participants revealed evidence of enrichment of exhausted and activated cells in the High group and naïve and memory CD28+ CD4+ T cells in the Low group pre-ART. Frequencies of anti-HIV CD8+ T cells were comparable between the groups; however, pre-ART innate immunity, as determined by the capacity of pDCs to produce IFN-α2 and the frequency of mature NK cells was significantly higher in the Low versus High group.

A number of previous studies have demonstrated that certain pre-ART parameters, such as plasma viremia, CD4+ T cell counts, CD4/CD8 T cell ratios, the timing of ART initiation, or T-cell activation and exhaustion are predictive of the HIV DNA burden in aviremic PWH receiving ART [13-22, 30, 31]. Our data corroborate previous findings and add new insight by employing several unique strategies. First, we employed additional molecular and cellular assays to delineate the impact of pre-ART variables on the levels of intact HIV DNA and inducible virion-associated HIV RNA. Second, we explored the role of pre-ART host innate immunity in determining the viral reservoir size following long-term ART. Lastly, we used a longitudinal approach to conduct in-depth analyses of the stability and composition of HIV DNA reservoirs over time.

Given that the vast majority of HIV DNA present in infected CD4+ T cells is replication-defective HIV DNA, we felt that it is important to address the role of pre- and post-ART parameters in the maintenance of persistent HIV reservoirs in PWH receiving ART by including quantitative measurements of intact proviral DNA. We found no significant differences in the ratio of intact to defective HIV DNA nor their decay rates between the High and Low groups. Additionally, the diversity and clonality of HIV env sequences in the total as well as subsets of
CD4+ T cells, were similar in both groups. These observations suggest that pre-ART elements rather than the modulation of infected CD4+ T cells during effective ART play a crucial role in shaping the size and the maintenance of the persistent HIV reservoirs that include infected CD4+ T cells carrying intact and inducible viruses.

Anti-HIV immune responses, especially those mediated by CD8+ T cells, have been shown to play a significant role in regulating virologic set points during the acute/early phase of infection [32-34]. However, the levels of immune exhaustion and polyfunctional HIV-Gag-specific CD8+ T cells in our study were comparable between the groups prior to the initiation of ART. It is plausible that the inclusion of other HIV antigens (such as Nef), the measurement of proliferative capacity of CD8+ T cells upon encountering antigens, and/or the employment of assays that directly measure the cytotoxic capacity of CD8+ T cells may have resulted in different outcomes.

pDCs and NK cells are important mediators of early innate immune response to HIV infection [25, 26] and thus may contribute to modulating the frequency of infected CD4+ T cells in PWH. We found that certain innate immune parameters, such as the capacity of pDCs to produce IFN-α2 upon CpG stimulation and the frequency of NK cells with higher cytotoxic potential differed between the two groups, and as such, may alter the size of HIV DNA reservoirs prior to initiation of ART. A previous study suggested that a robust innate immune response could potentially determine the outcome of latency-reversing agent on HIV reservoir size in PWH during ART [35]. In this regard, studies on early HIV infection demonstrated that the formation of the viral reservoir could be modulated by pDC/IFN-α activity [36, 37]. It has also been shown that pDCs can be productively infected by HIV, leading to their progressive loss and impaired function [38-40]. Moreover, loss of pDCs and IFN-α production has been described during acute HIV infection [41-43]. Nonetheless, we found comparable numbers of pDCs between the study groups prior to ART initiation, suggesting that preferential depletion of pDCs was an unlikely reason for the elevated level of HIV DNA in the High group. Rather, a functional defect in the pDCs of the High group may be contributing to the inefficiency in limiting the size of HIV reservoirs in PWH with high levels of HIV DNA.

The limitations of our study include the relatively small sample size, the absence of full genome sequencing of intact HIV proviral DNA, the lack of lymphoid tissue analyses, and the timing variability of post-ART measurements. Furthermore, it would be necessary to confirm our findings on the role of NK cells and pDCs in restricting the HIV DNA burden in PWH in a larger study involving longitudinal analyses. Nevertheless, our findings extend previous observations regarding the impact of pre-ART variables on the total HIV DNA in PWH receiving clinically effective ART with additional molecular and cellular assays that directly measure the intactness and/or replication competence of HIV reservoirs. Furthermore, our findings showing a potential role for innate immune cells in the modulation of HIV-infected cells offer novel therapeutic opportunities aimed at reducing the HIV reservoir size prior to and potentially during ART. Such
therapeutic interventions, together with other immune-enhancing agents, could potentially lead to ART-free virologic suppression in a subset of PWH.

NOTES

Author contributions. JB and TWC designed the research. JB, EJW, RS, VS, JSJ, MAR, BDK, MRM, LP, KG, SM and TWC performed the research. PAW contributed research material. JB, EJW, RS, CO, JL and TWC analyzed the data. JB, SM and TWC wrote the manuscript.

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References


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Table 1. Characteristics of study participants.

<table>
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<tr>
<th>Characteristic</th>
<th>High HIV DNA (n = 13)</th>
<th>Low HIV DNA (n = 15)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>Sex, number (%)</td>
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</tr>
<tr>
<td>Male</td>
<td>13 (100)</td>
<td>13 (87)</td>
<td>0.484&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Female</td>
<td>0 (0)</td>
<td>2 (13)</td>
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<tr>
<td>Age, years Median (interquartile range)</td>
<td>43 (32, 64)</td>
<td>46 (37, 56)</td>
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<td>Range</td>
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<tr>
<td>Race or Ethnic group, number (%)</td>
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<tr>
<td>African American</td>
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<td>5 (33)</td>
<td>0.140&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Unknown / multiple</td>
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<td>0 (0)</td>
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<td>Duration of HIV suppression on ART, years Median (interquartile range)</td>
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<td>Pre-ART plasma viremia (log&lt;sub&gt;10&lt;/sub&gt;copies/ml) Median (interquartile range)</td>
<td>5.02 (4.48-5.18)</td>
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<td>Pre-ART CD4&lt;sup&gt;+&lt;/sup&gt; T cell count (cells/µl) Median (interquartile range)</td>
<td>180 (89-321)</td>
<td>358 (238-492)</td>
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<td>Pre-ART CD8&lt;sup&gt;+&lt;/sup&gt; T cell count (cells/µl) Median (interquartile range)</td>
<td>1,087 (497-1,519)</td>
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<td>Pre-ART CD4&lt;sup&gt;+&lt;/sup&gt;/CD8&lt;sup&gt;+&lt;/sup&gt; T-cell count ratio Median (interquartile range)</td>
<td>0.18 (0.12-0.28)</td>
<td>0.45 (0.29-0.73)</td>
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<td>Range</td>
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<td>0.16-1.34</td>
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<tr>
<td>Post-ART Plasma viremia (copies/ml)</td>
<td>&lt;40</td>
<td>&lt;40</td>
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<td>Post-ART CD4&lt;sup&gt;+&lt;/sup&gt; T cell count (cells/µl) Median (interquartile range)</td>
<td>460 (336-572)</td>
<td>576 (450-857)</td>
<td>0.072</td>
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<td>Range</td>
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<td>594 (500-733)</td>
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<td>Post-ART CD4&lt;sup&gt;+&lt;/sup&gt;/CD8&lt;sup&gt;+&lt;/sup&gt; T-cell count ratio Median (interquartile range)</td>
<td>0.79 (0.57-1.02)</td>
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<sup>a</sup> Mann-Whitney test; <sup>b</sup> Fisher’s exact test
FIGURE LEGENDS

Figure 1: Comparison of virologic parameters between the High and the Low group. A) Levels of total HIV DNA, cell-associated HIV RNA, and inducible virion-associated HIV RNA in the High vs. Low group at the post-ART time point. B) Levels of intact, 5’defective, and 3’defective HIV DNA in the High vs. Low group at pre- and post-ART time points. C) Correlations between pre-ART CD4+ T cell counts (top row) or pre-ART plasma viremia (bottom row) and post-ART total, intact, 5’ and 3’defective HIV DNA, and inducible virion-associated HIV RNA. Red and blue symbols represent data points from the High and Low group, respectively. Triangles and circles represent pre- and post-ART time points, respectively. *P values were determined using the Mann-Whitney or the Wilcoxon matched-pairs signed rank test; **P < 0.001; ****P < 0.0001. Correlations were determined by the Spearman method.
Figure 1
Figure 2: Composition and dynamics of HIV DNA reservoir in total and CD4+ T cell subsets. A) Intact, 5’defective, and 3’defective HIV DNA decay rate in the High vs. Low group. The leftmost graph shows median values (HIV DNA copies/10^6 CD4+ T cells) for the High and Low group at 0.5, 2, 4, and 6-8 years on ART. The remaining graphs show comparisons of HIV DNA half-lives. Intact, 5’defective, and 3’defective proviruses are depicted in purple, orange, and green, respectively; the High group is in the dark, and the Low group is in light colors. B) Levels of Intact, 5’defective, and 3’defective HIV DNA in naïve (T_N), central memory (T_Cm), transitional memory (T_Tm), and effector (T_E) T cells. C) Comparison of CD4+ T cell subset frequencies between the High and the Low group. D) Cell contribution to the pool of intact HIV-infected CD4+ T cells. E) Comparison of HIV DNA sequence diversity calculated by Average Hamming Distance (left) and clonality (right) in total CD4+ T cells between the High and the Low group. F) Comparison of clonality among the four CD4+ T cell subsets and between the High and Low groups. High group samples are depicted in red, the Low group in blue, and the T cell subsets are as follows: T_N grey, T_Cm green, T_Tm orange, and T_E red. P values were determined using the Mann-Whitney or the Friedman test; *, P < 0.05, **, P < 0.01 *,*, P < 0.001; ****, P < 0.0001, ns, P ≥ 0.05.
Figure 2
Figure 3: Pre- and post-Art T-cell immune parameters in the High versus the Low group. 
A) Frequencies of the exhaustion (TIGIT and PD-1) and activation (CD38/HLA-DR) markers on CD4+ and CD8+ T cells in the High and Low groups pre- and post-Art. B-D) High-dimensional flow cytometric analysis. B) Uniform Manifold Approximation and Projection (UMAP) plots of CD3+ T cells from the High and Low group volunteers pre- and post-Art (left) and UMAP visualization of expression of the indicated markers (right). C) UMAP map of T cell clusters identified by FlowSOM clustering (left) and a heatmap showing the level of expression of individual markers in each cluster (right). Four out of the 15 clusters that showed significant differences between the groups are displayed. D) Comparison of frequencies of T cells expressing markers associated with indicated clusters: Cluster 2 (memory CD4+ T cells expressing CD28), Cluster 4 (transitional memory CD8+ T cells expressing CD38 and HLA-DR, and TIGIT, PD-1, and 2B4), Cluster 8 (naïve CD4+ T cells) and Cluster 9 (effector memory and terminally differentiated CD8+ T cells expressing CD226 and 2B4). E) Frequencies of polyfunctional HIV Gag-specific CD4+ (IFNγ+TNF-α+IL-2+CD40L+; left) and CD8+ (IFNγ+TNF-α+MIP-1β+; right) T cells in High and Low group pre- and post-Art. High group samples are depicted in red, Low group in blue, pre- and post-Art time points are depicted as triangles and circles, respectively. P values were determined using the Mann-Whitney and the Wilcoxon matched-pairs signed rank tests for unpaired and paired comparisons, respectively; *, P < 0.05, **, P < 0.01, ***, P < 0.001; ****, P < 0.0001, ns, P ≥ 0.05.
Figure 3
Figure 4: Innate immune characteristics in High and Low groups prior to initiation of ART.  

A) Frequencies of plasmacytoid dendritic cells (pDCs; left) and levels of IFNα-2 expression upon CpG stimulation of PBMCs per mL of cell-culture supernatant (right) pre- and post-ART.  

B) Frequencies of CD56dimCD16+ natural killer (NK) cells (left) and levels of CD57 expression in the CD56dimCD16+ NK cell compartment (right) in the High and Low group pre- and post-ART. High group samples are depicted in red, Low group in blue, and pre- and post-ART time points are depicted as triangles and circles, respectively. Light-colored symbols under the dotted line indicate values below the limit of detection. P values were determined using the Mann-Whitney test; *, P < 0.05, **, P < 0.01, ns, P ≥ 0.05.
Figure 4
Figure 5: Correlation between immunologic and virologic variables and their relation to HIV DNA reservoir size. Correlogram visualization shows the relationship between individual variables measured in this study in the High and Low group pre- and post-ART. Each row or column represents a different variable, ordered by the strength of the correlation with the post-ART HIV DNA levels. The size and color of each circle correspond to the correlation coefficient between a pair of variables, with negative values in red and positive in blue. The correlation coefficients were calculated using the Spearman method. Only significant correlations ($P < 0.05$) are shown.