Title

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Journal

AIDS, 34(5)

ISSN

0269-9370

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Publication Date

2020-04-01

DOI

10.1097/qad.0000000000002465

Peer reviewed

HHS Public Access

Author manuscript

AIDS. Author manuscript; available in PMC 2021 April 01.

Published in final edited form as:

AIDS. 2020 April 01; 34(5): 659–668. doi:10.1097/QAD.0000000000002465.

High levels of genetically-intact HIV in HLA-DR+ memory T-cells indicates their value for reservoir studies

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Abstract

Objective—The contribution of HLA-DR+ memory CD4+ T-cells to the HIV reservoir during prolonged antiretroviral therapy is unclear as these cells are commonly excluded when assessing for replication-competent HIV. To address this issue, we examined the distribution of genetically

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Author Contributions

B.A.H. and B.H conducted FLIPS on participant samples and analysed data; E.L conducted SGS on participant samples and analysed data; R.F conducted TILDA on participant samples and analysed data; R.H., S.D., F.H., E.S., J.M.M., S.v.S., L.O., N.C., and R. F. enrolled the participants, collected and/or sorted cell subsets from the participant samples; E.L., T.L., S.v.S., L.O., E.A.B., and D.C.D. prepared participant samples; J-S.E. designed analysis and data visualization workflows; T.E.S. conducted the statistical analyses. B.A.H. wrote the original manuscript; S.P. designed the study, supervised the work performed, and edited the manuscript.

Conflict of Interest Statement

The authors declare that no conflict of interest exists.

intact HIV DNA within HLA-DR- and HLA-DR+ memory $CD4^+$ T-cells and the RNA transcriptional profile of these cells during ART.

Design/Methods—Full-length DNA sequencing was used to examine the HIV DNA landscape within HLA-DR+ and HLA-DR- memory CD4+ T-cells. RNA quantification and sequencing was used to interrogate the relationship between HLA-DR status and HIV RNA transcription.

Results—HLA-DR+ CD4+ T-cells contained a high frequency of genetically-intact HIV genomes, contributing over half of the genetically-intact viral sequences to the reservoir. Expansions of genetically identical sequences were identified in all T-cell subsets, indicating that cellular proliferation maintains genetically-intact and defective viral DNA during therapy. Intracellular HIV RNA levels in HLA-DR+ and HLA-DR- T-cells were not statistically different by either LTR qPCR quantification or single-genome RNA sequencing of the p6-RT region.

Conclusions—The high proportion of intact viral DNA sequences in the proliferative HLA-DR + subset suggests they are critical in maintaining HIV infection during effective therapy. As such, these cells should be included in any immune intervention targeting HIV during effective therapy.

Keywords

HIV Reservoir; Full-Length Sequencing; Genetically-Intact HIV; HLA-DR+ Memory Cells

Introduction

Despite continued viral suppression, replication-competent HIV remains in long-lived memory CD4+ T-cells in participants on long-term anti-retroviral therapy (ART) [1–4]. This latent virus is the main barrier to a cure as cells containing replication-competent HIV can reinitiate infection if therapy ever ceases. A clear understanding of the cellular source and mechanisms contributing to the maintenance of this reservoir will be key to developing a targeted cure.

Two methods to measure persistent HIV have been developed; PCR-based assays, and culture-based assays such as the Quantitative Viral Outgrowth Assay (QVOA) [5]. There is little correlation between these methods [6]. QVOA is known to underestimate the size of the reservoir as not all proviruses are induced after a single round of stimulation [7]. By contrast, PCR-based assays provide an overestimate as they can detect defective HIV genomes [7, 8]. Another reason for inconsistent results could be the exclusion of CD4+ T-cells expressing HLA-DR from the QVOA, on the assumption that viral production occurs in these cells.

HLA-DR expression is correlated with immune checkpoint molecules expressed on cells enriched for HIV [9], such as PD-1 [9–11] and TIGIT [9, 11, 12]. Expression of HLA-DR is correlated to the level of total HIV DNA in resting CD4+ T-cells contributing to HIV persistence [13, 14]. HLA-DR is also upregulated on effector memory CD4+ T-cells [15], which have been shown to contain a high frequency of genetically-intact and translation-competent viral DNA [8, 11]. HLA-DR+ cells, therefore, are an important yet understudied cell population.

Proliferation of the host cell, either by homeostatic mechanisms or through antigen recognition, has been shown to maintain both intact and defective proviruses [16–21]. However, proliferation of HIV-infected cells has not been specifically assessed in the HLA-DR+ subset. We therefore examined proliferation in HLA-DR+ cells to determine how this may impact the maintenance of HIV-infected cells.

Furthermore, the levels of endogenous intracellular HIV RNA in HLA-DR+ cells, and the ability of these cells to release virus when stimulated, is unknown. Assessing their contribution to active viral replication during therapy would further delineate the importance of including HLA-DR+ cells in any immune therapy targeting the latent reservoir.

In this study, we used the full-length individual proviral sequencing (FLIPS) assay to examine memory T-cell subsets, including those expressing HLA-DR, for the presence of genetically-intact and likely replication-competent virus. HLA-DR+ cells contained a high frequency of intact HIV genomes. Expansions of genetically identical sequences are seen in all participants and T-cell subsets, but are enriched in HLA-DR+ cells. There is no significant difference in the endogenous level of HIV RNA within HLA-DR+ and HLA-DR- cells. As such, we find that HIV infection is maintained within both HLA-DR+ and HLA-DR- memory T-cells by cellular proliferation. HLA-DR+ cells should therefore be included in assays aimed at measuring persistent HIV, and will be a key target in any immune intervention designed to limit the reservoir.

Methods

Study Approval

Written informed consent was obtained from all participants. This study was approved by the institutional review board at the Western Sydney Health Department for the Westmead Institute for Medical Research, and the ethics review committees at the University of California San Francisco and Vaccine & Gene Therapy Institute-Florida.

Participants

Leukapheresis samples were obtained from six HIV-1 subtype-B positive individuals on long-term ART (Table 1). Three participants initiated therapy during chronic (>1 year) infection, and three during acute/early (<6 months) infection. All participants had been on therapy >3 years; the four participants with HLA-DR+ and HLA-DR- cells (SCOPE2026, SCOPE2046, SCOPE2115, SCOPE2275) had been on therapy >15 years.

Cell Sorting

Fluorescence-activated cell sorting (FACSAria [BD Biosciences]) was used to sort naïve, central memory (CM), transitional memory (TM) and effector memory (EM) CD4+ T-cell subsets as previously described [8, 17, 19] (Table 1). CD4+CD45RA-HLA-DR+ and CD4+CD45RA-HLA-DR- memory T-cells were sorted to high purity (>99%) from the same leukapheresis from four participants (Table 1, Supplementary Methods, Figure S1).

FLIPS Assay

The FLIPS assay was performed as previously reported [8], and viral sequences were identified as intact or defective [8]. Genbank accession numbers for FLIPS sequences are MH843739-MH843916, KY778264-KY778681 and KY766150-KY766212.

Further Analysis of Sequences

The V3 loop was analysed for predicted tropism using the X4R5 matrix for subtype B sequences, Web PSSM (https://indra.mullins.microbiol.washington.edu/webpssm/) [22].

Expansions of identical sequences (EIS) were identified using Elimdupes (Los Alamos HIV Database). Sequences that were 100% identical belonged to the same EIS. Maximum-likelihood trees were drawn in MEGA6 [23] to confirm the presence of EIS. A general time-reversible model, gamma-distributed with invariant sites (category 4) method was used, with 500 bootstrap replicates. Annotated tree images were constructed using ggtree [24].

HIV infection frequencies were determined using a logistic model (Supplementary Methods).

Genetic diversity calculations were performed using pairwise distance as calculated in MEGA6.

HIV RNA quantification by real-time RT-PCR

Intracellular HIV RNA levels were measured using RT-PCR as previously reported [25].

HIV RNA Quantification and Comparisons by Single-Genome Sequencing

Single-genome sequencing (SGS) was performed on the gag-pol p6-RT region as previously described [17, 19, 26, 27]. The p6-RT region was extracted from FLIPS-derived DNA sequences and aligned with the SGS-derived sequences, and identity at the 100% and >99% levels determined (ElimDupes, Los Alamos HIV Database). Transcripts that matched defective or intact genomic sequences were compared using a t-test (Stata 15, StataCorp. 2017). Genbank accession numbers for SGS sequences are MK473661-MK473751.

Tat/Rev Limiting Dilution Assay

The Tat/Rev Limiting Dilution Assay (TILDA) was performed as previously reported [28] (Table S1).

Results

We obtained naive, CM, TM and EM CD4+ T-cells from a leukapheresis from six participants on suppressive ART (Table 1). Memory HLA-DR+ and HLA-DR- CD4+ T-cells were additionally sorted from four participants (Table 1, Figure S1). HLA-DR+ cells displayed a higher level of Ki67 and CD38 ($p<0.001$), CCR5, CD71 and PD-1 ($p<0.01$), and Tim3 ($p<0.05$) than HLA-DR- cells. We used the FLIPS assay [8] to sequence near full-length (92%) HIV genomes at the single-genome level. A total of 732 viral sequences were isolated, and the majority of sequences (64%) contained a large internal deletion (Figure 1a).

Thirty-seven sequences (5%) were genetically-intact (Figure 1a, Table S2). Ninety-two percent of all sequences were predicted to use the CCR5 co-receptor, with 93% of defective and 83% of intact sequences predicted to be CCR5-tropic (Figure S2a).

From the HLA-DR+ subset, 98 sequences were isolated from approximately 640 000 cells (121142–196379 per participant) (Table S2). Of these, seven (7.1%) were genetically-intact (Figure 1a, Figure S3). The majority (51%) of sequences contained a cis-acting mutation; these sequences had defects in the stem loops or major splice donor site (MSD) (Figure 1a, Figure S3) [7]. The HLA-DR+ subset had a high proportion of genomes with genetically-intact *tat* (Trans-Activator of Transcription) (Figure S4). HLA-DR+ cells also contained a high proportion of CCR5-tropic sequences (Figure S2b).

Within the HLA-DR- cell subset, 103 sequences were isolated from approximately 3 million cells (55790–1910920 per participant) (Table S2), and, four (3.9%) were genetically-intact (Figure 1a). The majority (69%) of sequences contained a large internal deletion, contrasting to HLA-DR+ cells (33% of sequences with deletions) but comparable to the pooled data from all cell subsets (64% of sequences with deletions) (Figure 1a, Figure S3).

HLA-DR+ cells have a higher HIV infection frequency than HLA-DR- cells

To determine how HLA-DR+ cells contribute to the persistent reservoir during therapy, we first determined if the total infection frequency with viral DNA, was different between HLA-DR+ and HLA-DR- CD4+ T-cells. We found the infection frequency of HLA-DR+ cells was 216 cells per 10^6 cells (range 41–314), and HLA-DR- cells was 193 cells per 10^6 cells (range 23–583) (Table S3). There was evidence in all participants that the infection frequencies of these subsets was different ($p<0.001$, Fisher's exact test), with HLA-DR+ cells demonstrating a higher infection frequency than HLA-DR- cells in all participants except SCOPE2046 (Figure 1b).

The highest infection frequency with genetically-intact viral DNA is found in HLA-DR+ and EM CD4+ T-cells

As understanding the contribution of replication-competent proviruses from each T-cell subset is important when targeting HIV curative strategies, we calculated the infection frequency of genetically-intact and likely replication-competent viral DNA in all cell subsets.

When comparing HLA-DR+ and HLA-DR- cells, we found HLA-DR+ cells to contain a higher proportion of intact viral DNA, though this was not significant ($p=0.25$, Wald test) (Figure S5a). We found that the intact infection frequency was significantly different across all memory cell subsets ($p<0.001$, likelihood ratio test) (Figure S5b). The infection frequencies within each T-cell subset were also different between participants ($p<0.001$, likelihood ratio test), however there was no evidence that the way the infection frequency varied across the T-cell subsets differed between participant (effect modification $p=0.38$, likelihood ratio test) (Figure S5b). This indicates that despite the differences in infection frequencies overall, our observed pattern of infection with an intact virus was similar for each participant. In HLA-DR+ cells, an average of 13 cells per 10^6 cells contained intact viral DNA (range 0–23) (Table S3). When intact HIV genomes were found in the HLA-DR+

subset, HLA-DR+ cells had a higher intact infection frequency than HLA-DR- cells (Figure 1c). HLA-DR- cells had an average intact infection frequency of 2 cells per 10^6 cells (range 0–8) (Table S3), and as such a 6.5-fold higher infection frequency was observed in HLA-DR + cells (p=0.018, Fisher's exact test) (Figure 1d). Although they form only 11% of memory CD4+ T-cells (Table S4), HLA-DR+ cells contributed over half (56%) of all genetically-intact viral sequences to the memory T-cell reservoir (Figure S5c).

When compared to cell subsets studied previously [8], one participant (SCOPE2115) had the highest infection frequency in HLA-DR+ cells (Figure S5b). In the other participants, HLA-DR+ cells had a higher infection frequency than CM and TM, but not EM cells (Figure S5b). In one participant (SCOPE2026) the same genetically-intact sequence was identified in TM, EM, HLA-DR+ and HLA-DR- cells. No other intact sequence was 100% identical between the EM and HLA-DR+ cell subsets, despite the infection frequencies being similar in these subsets (Figure S6).

HLA-DR+ T-cells contain the most expansions of identical HIV sequences

Cellular proliferation can occur when homeostatic signals stimulate proliferation to retain a stable memory cell pool [29], or when a cell recognises its cognate antigen and undergoes a massive proliferative event [30]. Most studies of the proliferation of HIV-infected cells are performed by sequencing a small region of the genome [16, 17, 19], by stimulating cells ex-vivo [16, 31] or by sequencing the proviral integration site [32, 33]. Our study allows us to infer cellular proliferation by observing sequences that are 100% identical across the full-length genome. Such identical sequences are most likely to arise due to proliferation of the host cell, however they could also represent a homogenous viral population at the time of ART initiation.

Expansions of identical sequences (EIS) were identified in all participants and all subsets (Figure 2, Figure S7). There was no difference in the proportion of intact or defective sequences belonging to an EIS (p=0.17, Fisher's exact test) (Figure 2a). There was evidence, however, that the proportion of sequences which contributed to an EIS varied across memory subsets (p<0.001, likelihood ratio test); that is, while all memory cell subsets had proliferated, their capacity to proliferate was different (Figure 2b). The way this varied was different between participants (effect modification p<0.0001, likelihood ratio test), as was the percentage of sequences contributing to an EIS within each participant (p<0.001, likelihood ratio test). This was expected, as the mechanisms contributing to these expansions would be influenced by clinical history. Three participants had a higher proportion of sequences that were part of an EIS in HLA-DR+ cells compared to HLA-DR- cells (p<0.001–0.003, Fisher's exact test) (Figure 2c). We did not observe any difference in SCOPE2046 (p=0.39, Fisher's exact test) (Figure 2c), which may reflect the low number of sequences derived from HLA-DR+ cells (n=6) in this participant. Genetic diversity calculations confirmed that viral DNA isolated from HLA-DR+ cells had low genetic diversity, indicative of high levels of identical sequences (Table S5, Figure S8).

Levels of intracellular HIV RNA are similar between HLA-DR+ and HLA-DR- cells

HLA-DR+ cells are not analysed in studies of the latent reservoir as they are assumed to spontaneously produce HIV RNA [34]. We therefore quantified cell-associated HIV RNA using primers located in the LTR region without external stimulation [25] to determine if these cells do spontaneously produce HIV RNA. HLA-DR+ cells contained more HIV RNA transcripts than HLA-DR- cells (p=0.051, two-sample T test) (Figure 3a). This may reflect the higher infection frequency in HLA-DR+ cells, so we compared the ratio of HIV RNA to viral DNA in both cell subsets. When normalised to the HIV DNA level, HIV transcriptional activity in the HLA-DR+ and HLA-DR- cell subsets was similar (p=0.35, two-sample T test) (Figure 3b).

We then interrogated the nature of this RNA by performing single-genome sequencing (SGS) of the gag-pol region (p6 through nucleotides 1–900 of the gene encoding reverse transcriptase, p6-RT). SGS quantification may be more specific for actively transcribing virus, as assays that utilize the LTR region may be affected by cellular read-through, or may represent transcription that does not result in an elongated transcript [35]. When normalized by the cell numbers used, the levels of p6-RT transcripts were comparable between HLA-DR+ and HLA-DR- cells (Table 2, p=0.73, t-test). We note that we were able to sequence HIV RNA from HLA-DR+ cells in one participant only.

We then compared the HIV transcripts to viral sequences obtained by FLIPS to determine whether cell-associated p6-RT RNA is transcribed from intact and/or defective genomes. We compared RNA and DNA sequences at both 100% and >99% genetic identity using ElimDupes (Table 2) (http://www.hiv.lanl.gov). Greater than 99% identity allowed for up to 11 nucleotide changes due to possible assay related errors [26, 36]. For the HLA-DR+ subset, no HIV RNA sequence was 100% identical to an intact DNA sequence. However, 20 HIV RNA sequences in 10^6 cells were >99% identical to both defective and intact HIV genomes. For the HLA-DR- subset, the average number of HIV RNA sequences per 10^6 cells that were 100% identical to an intact or defective DNA sequence was similar (Table 2, p=0.74). However, 4-fold more HIV transcripts per 10^6 cells were >99% identical to defective sequences compared to intact (Table 2, p=0.19).

The Tat/Rev Limiting Dilution Assay (TILDA) was performed on HLA-DR+ and HLA-DR- cells from SCOPE2275 to assess RNA production both with and without external stimulation. Without stimulation, 1.8 cells per 10^6 HLA-DR- cells produced multi-spliced (ms) RNA, and the number of msRNA producing cells in the HLA-DR+ subset was below the limit of detection, 5.7 msRNA producing cells per 10^6 cells (Figure S9). When the cells were stimulated with PMA/ionomycin, a 3.5 fold higher number of HLA-DR+ cells expressed msRNA than did HLA-DR- cells (Figure S9).

Together, this indicates that unstimulated viral transcription is not specific to genetically-intact HIV or the HLA-DR+ cell subset. The lack of any significant difference in endogenous viral transcription in HLA-DR+ and HLA-DR- cells indicates that the singular HLA-DR marker is not a surrogate for cells actively producing HIV RNA during effective therapy.

Discussion

In our study, cells expressing HLA-DR were found to have a higher infection frequency with intact viral DNA compared to HLA-DR- cells. HLA-DR+ cells did not endogenously produce more HIV RNA than HLA-DR- cells, however after stimulation they produced msRNA to a higher level. Our findings highlight the importance of HLA-DR+ cells in harbouring 56% of the genetically-intact and likely replication-competent virus during effective ART. As such, these cells should be included in any immune intervention aimed at limiting the HIV reservoir.

HLA-DR+ cells contained the highest proportion of identical sequences, indicative of cellular proliferation [21]. There was no difference in the proportion of genetically identical intact and defective sequences, indicating that cellular proliferation is not causing virion release. If virions were released during proliferation lower levels of genetically-intact identical sequences may be expected, as these cells would be targeted by the immune system or killed by viral cytopathic effect. This finding is supported by work showing that homeostatic proliferation increases the frequency of integrated provirus but does not increase viremia [29]. The maintenance of genetically-intact HIV by cell proliferation has been observed in other cell subsets, such as T_H1 cells [20]. HLA-DR+ cells are historically thought of not only as highly proliferative, but also as short-lived. However, a recent longitudinal study examining viral gag-pol DNA sequences within HLA-DR- and HLA-DR + memory CD4+ T-cell subsets revealed that HIV infection frequencies in HLA-DR+ T-cells are stable, even increasing, over 3–17 years of therapy [37]. This study also found that sequences persisted over two years within the HLA-DR+ subset, with 33% of sequences being observed at multiple time-points during therapy [37]. Longitudinal studies of genetically-intact HIV within the HLA-DR+ subset would further allow us to assess the persistence of these genomes, and whether cellular proliferation maintains these genetically-intact genomes continuously during therapy.

The quantification of HIV RNA LTR transcripts by qPCR indicates that short read-through transcription occurs in both HLA-DR+ and HLA-DR- subsets [38–41]. These read-through host-HIV transcripts can enhance HIV gene expression, potentially elevating cellular activation [32, 33, 42]. However, our findings indicate that cells expressing HLA-DR are only partially activated, as stimulation of HLA-DR+ cells was required to produce tat/rev msRNA above the limit of detection, though we do note that given the physiological make-up of memory cells in the body the cell input for HLA-DR+ cells in this assay was small. These results do agree with the fact that we were able to sequence p6-RT RNA from the HLA-DR+ T-cells of only one participant, however. The scarcity of p6-RT RNA sequences suggests a block in transcriptional elongation beyond the 5’ LTR [35]. This indicates that possible transcriptional interference in HLA-DR+ cells may contribute to HIV persistence, despite their activation status and high levels of genetically-intact *tat*.

Previous studies have used intracellular HIV RNA as a marker of the active viral reservoir [38, 43–45]. However, as viral RNA can be produced from both defective and intact HIV genomes, this is not an adequate biomarker, and its use will lead to an overestimation of the genetically-intact HIV reservoir.

As HLA-DR+ cells are not necessarily actively infected, we propose instead that it is the integration of full-length provirus that is pushing the cell into a more activated state. Defective viral DNA has been shown by ourselves and others to produce RNA [46] and activate killing by the immune system [47, 48]. Perhaps this RNA and protein is also recognised intracellularly, leading to self-activation. While the amount of RNA being produced by HLA-DR+ and HLA-DR- cells was not different, both cell subsets contain distinct templates for RNA production. As an example, HIV genomes that contain cis-acting mutations are more common in the HLA-DR+ cell subset, and such sequences may be an ideal template for the production of immunogenic RNA. It is plausible that the integration of a more intact provirus, and subsequent low level RNA production from this template, is driving these cells to exist in a partially activated state.

The sample size was low for this study, which in turn limited the number of sequences isolated. While a greater number of participants may strengthen our findings, it is worth noting that our data does reach statistical significance. Furthermore these findings are not surprising as previous work has shown that integrated HIV DNA is associated with the level of HLA-DR+ cells [13], and that cells containing CCR5-tropic virus express higher levels of HLA-DR [49]. Other activation [50] and exhaustion markers [9] have also been shown to enrich for HIV infected cells, as have EM cells [8, 11], a cell subset which correlates with HLA-DR expression [15].

Correspondingly, we also found HLA-DR+ memory cells to display a higher frequency of exhaustion markers than their HLA-DR- counterparts. As such, latency reversing agents which rely on T-cell activation to stimulate viral production may not be effective in these cells, as exhausted CD4+ T-cells may be less responsive to stimulation as they display high levels of inhibitory receptors [51]. However, immunotherapies, such as immune checkpoint inhibitors, which act against activation and exhaustion markers have shown some efficacy in reversing HIV latency [52–54]. The concentration of intact HIV in activated and exhausted cells, such as HLA-DR+ cells, therefore, raises the possibility that immunotherapeutic agents may be a successful HIV curative strategy in these cells.

HLA-DR is not a marker of a virion-producing cell, but rather is present on cells with a high proportion of intact viral sequences and with a high proliferative potential. To prevent the contribution of subsets such as HLA-DR+ CD4+ memory T-cells from being overlooked, assays of the reservoir should include all cells with the potential to produce infectious virus, regardless of any assumptions regarding activation status. As an alternative, a greater understanding of the level of activation which leads to viremia will also allow for the development of standardised assays of the latent reservoir. The accurate identification of any cell which could produce infectious virus will be key to the targeting and assessment of any immune intervention for the elimination of these persistent HIV-infected cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Delaney AIDS Research Enterprise (DARE) to Find a Cure (1U19AI096109 and 1UM1AI126611-01), an amfAR Research Consortium on HIV Eradication (ARCHE) Collaborative Research Grant from The Foundation for AIDS Research (amfAR 108074-50-RGRL), the UCSF/Gladstone Institute of Virology & Immunology Center for AIDS Research (P30 AI027763), Australian Centre for HIV and Hepatitis Virology Research (ACH2) (2015-Joey Lai, Genomics Facility Manager at The Westmead Institute for Medical Research for the use of his facility, the Sydney Informatics Hub (University of Sydney, Sydney, Australia) for support for and access to analytical software, and the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia) for operating the Miseq. B.A.H would like to thank J.W. Rhodes and K. Fisher for their support and insights on the manuscript, and O. Tong for helpful discussions. We acknowledge with gratitude the participants who donated samples for this study.

Funding

This work was supported by the Delaney AIDS Research Enterprise (DARE) to Find a Cure (1U19AI096109 and 1UM1AI126611-01), an amfAR Research Consortium on HIV Eradication (ARCHE) Collaborative Research Grant from The Foundation for AIDS Research (amfAR 108074-50-RGRL), the UCSF/Gladstone Institute of Virology & Immunology Center for AIDS Research (P30 AI027763), Australian Centre for HIV and Hepatitis Virology Research (ACH2) (2015-43), and the Australian National Health and Medical Research Council (APP1061681 and APP1149990).

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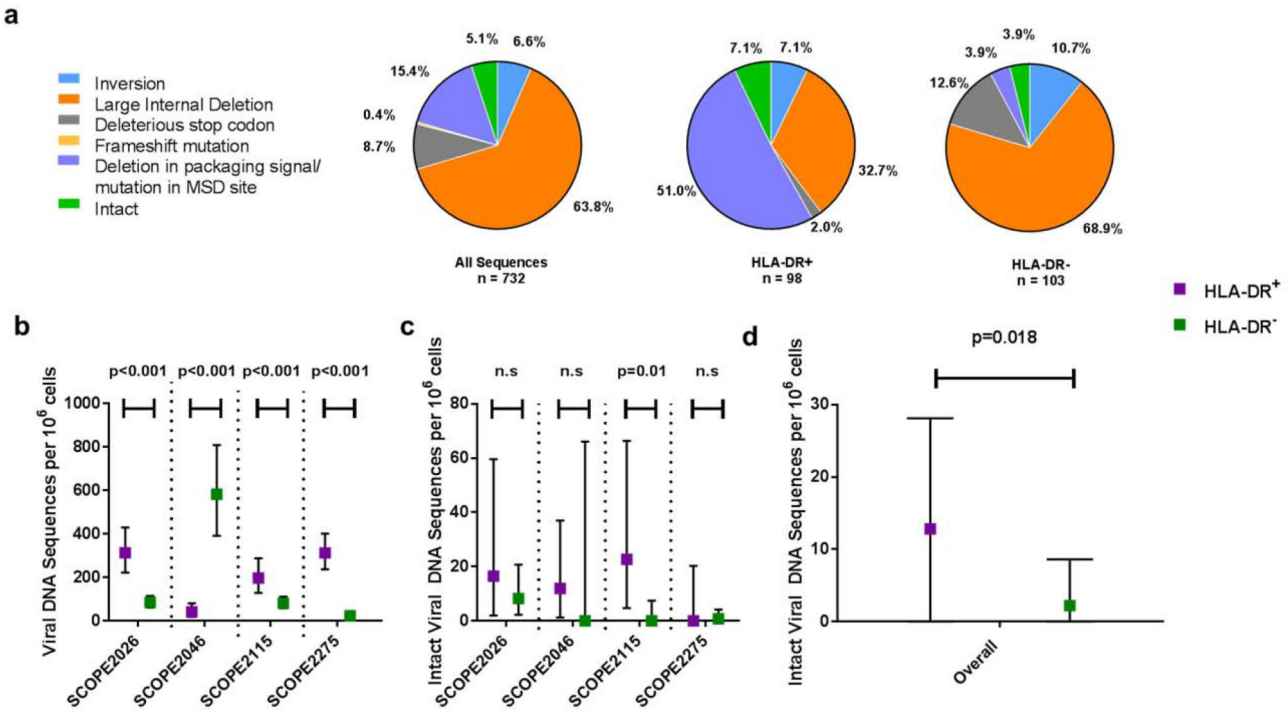

Figure 1. The contribution of HLA-DR+ and HLA-DR- memory CD4+ cells to the pool of HIV infected cells.

(**A**) Percentage of defective and intact viral DNA sequences isolated from all cell subsets (HLA-DR+ and HLA-DR- memory CD4+ T-cells, central, transitional and effector memory CD4+ T-cells, naïve CD4+ T-cells) (**B**) Number of viral DNA sequences per million cells. Fisher's exact test, data is mean ± 95% CI. (**C**) Number of intact HIV sequences per million cells. Fisher's exact test, data is mean ± 95% CI. (**D**) Overall infection frequency with an intact viral DNA sequence in n=4 participants. Fisher's exact test, data is mean ± 95% CI.

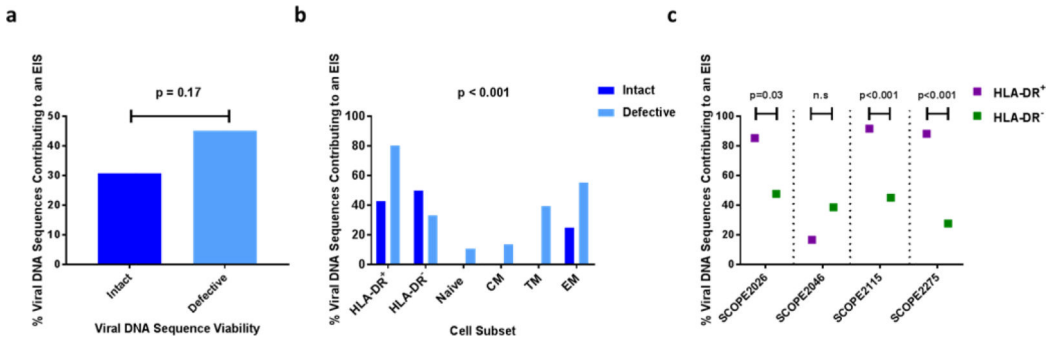

Figure 2. Expansions of identical sequences within the latent reservoir.

(**A**) The percentage of intact and defective viral sequences contributing to an expansion of identical sequences (EIS). Fisher's exact test. Data pooled from all participants. (**B**) The percentage of isolated sequences from various cell subsets contributing to an EIS. Data included from four participants with HLA-DR+/− cells, and six participants with N, CM, TM and EM T-cells. Strong evidence for effect modification ($p<0.0001$, likelihood ratio test) also present. (**C**) The percentage of sequences contributing to an EIS from HLA-DR+ and HLA-DR- memory CD4+ T-cells. Fisher's exact test.

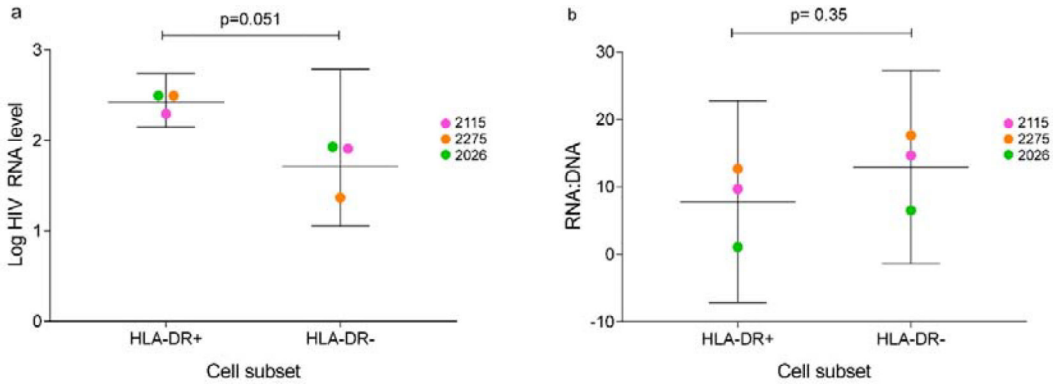

Figure 3. The levels of intracellular HIV RNA in HLA-DR+ and HLA-DR- cells.

(**A**) Intracellular HIV RNA copies per 10^6 cells as measured by qPCR using primers located in the LTR region. Two-sample t-test. Data is mean ± 95% CI. HIV RNA levels were log transformed. (**B**) The ratio of HIV LTR RNA levels measured by qPCR to HIV DNA levels quantified using FLIPS. Two-sample t-test. Data is mean ± 95% CI.

Table 1.

Participant Characteristics.

Participant (SCOPE ID)	Sex	Age	Viral load (copies/mL)	CD4+ T-cell count (cells per μL)	Time of infection before initiation of therapy (months)	ART duration (years)	Therapeutic regimen	Cell Subsets Available
2302	male	27	<40	696	<6	4.6	FPV,RTV, TDF/FTC	Naïve, CM, TM, EM
2115	male	51	<40	601	<6	17.3	FTC/TDF,NVP	HLA-DR+, HLA-DR-, CM, TM, EM
2275	male	47	<40	1842	<6	15.3	FTC/TDF,NVP	HLA-DR+, HLA-DR-, CM, TM, EM
2452	male	66	<40	604	>12	3.2	MVC,RTG,ETR	Naïve, CM, TM, EM
2026	male	59	<40	476	>12	17.7	TDF,ABC/3TC,RTV,DRV	HLA-DR+, HLA-DR-, Naïve, CM, TM, EM
2046	male	50	<40	1099	>12	16.3	ECV, EFV/TDF/FTC	HLA-DR+, HLA-DR-, Naïve, CM, TM, EM

Viral Load, CD4+ T-cell count and ART duration are all as at the time of sampling. FPV, fosamprenavir; RTV, ritonavir; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; NVP, nevirapine; MVC, maraviroc; RTG, raltegravir; ETR, Etravirine; ABC, abacavir; 3TC, lamivudine; DRV, darunavir; EFV, efavirenz; ECV, entecavir; CM, Central Memory; TM; Transitional Memory; EM, Effector Memory

Table 2.

Quantification and genetic characterization of HIV p6-RT RNA sequences

Participant (SCOPE ID)	No. of cells used for SGS	No. of p6-RT RNA sequences	No. of p6-RT RNA sequences/ 10^6 cells	No. of p6-RT RNA sequences matching defective HIV genome/ 10^6 cells		No. of p6-RT RNA sequences matching intact HIV genome/ 10^6 cells	
				100% identity	99% identity	100% identity	99% identity
RNA from HLA-DR+ Cells							
2275	355,259	0	0 (<3)[b]	n/a	n/a	n/a	n/a
2115	200,000	10	50	0	20	0	20
2026	137,500	0	0 (<7)[b]	n/a	n/a	n/a	n/a
2046	7,875	0	0 (<127)[b]	n/a	n/a	n/a	n/a
Average[a]	175,159	3	12.5	n/a	n/a	n/a	n/a
	[−54,432, 404,750]	[−5, 10]	[−27.3, 52.3]				
RNA from HLA-DR- Cells							
2275	3,367,111	13	3.9	2.1	3.9	1.5	3.9
2115	2,300,000	29	12.6	0	2.6	0	2.6
2026	3,549,310	38	10.7	0.3	6.2	0	8.2
2046	22,125	1	45.2	0	45.2	0	0
Average[a]	2,309,636	20	18.1	0.6	14.5	0.4	3.7
	[−270,541, 4,889,814]	[−6, 46]	[−11.3, 47.5]	[−1.0, 2.2]	[−18.2, 47.2]	[−0.8, 1.6]	[−1.8, 9.1]

[a] Lower and upper 95% confidence intervals are shown in square brackets. Average calculated based on absolute numbers.

[b] Limit of detection based on number of cells used.