

Clonality of HIV-1 and HTLV-1 infected cells in naturally coinfecting individuals

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Summary of main points

We investigated the mutual effect of HIV-1/HTLV-1 coinfection on their integration sites and the clonal expansion and found the degree of oligoclonality of both HIV-1- and HTLV-1-infected cells in co-infected individuals was greater than that in mono-infected subjects.

We obtained written permission for all personal communications

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Abstract

Background:

Coinfection with HIV-1 and HTLV-1 diminishes the value of the CD4⁺ T-cell count in diagnosing AIDS, and increases the rate of HTLV-1-associated myelopathy. It remains elusive how HIV-1/HTLV-1 coinfection is related to such clinical characteristics. Here, we investigated the mutual effect of HIV-1/HTLV-1 coinfection on their integration sites (ISs) and the clonal expansion.

Methods:

We extracted DNA from longitudinal peripheral blood samples from 7 HIV-1/HTLV-1 coinfecting individuals, and from 12 HIV-1 and 13 HTLV-1 mono-infected individuals. The proviral loads (PVL) were quantified using real-time PCR. Viral ISs and clonality were quantified by ligation-mediated PCR followed by high-throughput sequencing.

Results:

The PVL of both HIV-1 and HTLV-1 in coinfecting individuals was significantly higher than that of the respective virus in mono-infected individuals. The degree of oligoclonality of both HIV-1- and HTLV-1-infected cells in co-infected individuals was also greater than that in mono-infected subjects. The ISs of HIV-1 in cases of coinfection were more frequently located in intergenic regions and transcriptionally silent regions, compared with HIV-1 mono-infected individuals.

Conclusion:

HIV-1/HTLV-1 coinfection makes an impact on the distribution of viral ISs and the clonality of virus-infected cells and thus may alter the risks of both HTLV-1- and HIV-1-associated disease.

Keywords:

HIV-1, HTLV-1, coinfection, Integration site analysis, High-throughput sequencing

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Introduction

Human immunodeficiency virus 1 (HIV-1) and human T-cell leukemia virus type 1 (HTLV-1) are structurally similar retroviruses that infect humans, but present a dramatically different natural history and clinical features. HIV-1 infects the target cells via CD4 and chemokine receptors, while HTLV-1 does so via GLUT-1, neuropilin and heparan sulfate proteoglycan receptors [1-5]. After infection, their viral RNA genomes are reverse-transcribed to a double-stranded DNA which is then integrated into the host genome to form the provirus. HIV-1 infects CD4⁺ T-cells and causes acquired immunodeficiency syndrome (AIDS). Although combined antiretroviral therapy (cART) can effectively reduce the amount of virus and has remarkably improved patients' lifespan, HIV-1 can persist in a latent state as an integrated provirus in various cell types, including resting memory CD4⁺ T-cells. HTLV-1 causes a chronic infection after transmission through breastfeeding or sexual intercourse. Although the majority of HTLV-1-infected individuals are asymptomatic carriers, the virus sporadically causes adult T-cell leukemia-lymphoma (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Unlike HIV-1, antiretroviral treatment is not effective in HTLV-1 infection.

A retrovirus-infected clone is identified by the unique integration site of the provirus in the host genome. Previous reports revealed that both HIV-1 and HTLV-1 preferentially target euchromatin, whose open chromatin conformation allows the retroviruses access to the host DNA [6, 7]. The clonality of virus-infected cells can be quantified by identifying the proviral integration site by next-generation sequencing. Clonal proliferation of HIV-1-infected cells is caused by some drivers including antigen-driven proliferation and the viral integration site-dependent proliferation [8] [9] [10] [11]. In contrast, the factors that determine clonal proliferation of HTLV-1-infected cells include the expression of viral proteins, Tax and HTLV-1 bZIP factor, and the viral integration site [12, 13][14]. Malignant transformation of HTLV-1-infected cells to ATL is caused by both driver genetic mutations and epigenetic modifications in the host cell genome [15] [16].

Screening of HTLV-1 infection was performed in several HIV-1 cohort studies which reported the prevalence of HIV/HTLV coinfection. In Rio de Janeiro, an endemic area for HTLV-1, coinfecting patients accounted for 10.9% of HIV-infected individuals, and HIV-1/HTLV-1 coinfection appeared to affect the clinical manifestations of each viral infection [17]. One prospective cohort and two retrospective studies have shown that HIV-1/HTLV-1 coinfecting patients had a significantly higher CD4⁺ T-cell count than HIV-1 mono-infected patients at baseline [18] [19] [20]. Furthermore, there was a discrepancy between the CD4⁺ T-cell count and the immunocompetence status, making the CD4⁺ T-cell count a poor surrogate marker for starting cART in HIV-1/HTLV-1 coinfecting patients. Regarding the effect of HIV-1 coinfection on HTLV-1-associated diseases, prospective observational studies showed a higher incidence of HAM/TSP in the coinfecting patients' cohorts than the mono-infected ones (15.7% and 9.7%) [21, 22].

These previous findings indicate that HIV-1/HTLV-1 coinfection might influence the natural history and pathogenesis of each individual infection. We used a quantitative high-throughput sequencing approach to test the hypothesis that coinfection with HIV-1 and HTLV-1 alters the distribution of viral integration sites and the clonality of both HIV-1- and HTLV-1-infected cells.

Methods

Clinical samples

Patients attended the National Centre for Human Retrovirology (Imperial College Healthcare NHS Trust, St Mary's Hospital, London) or the department of Infectious Control at Kumamoto University, and donated blood samples, after giving a written informed consent in accordance with the Declaration of Helsinki. This study was approved by the UK National Research Ethics Service (NRES: ref. 15/SC/0089) and the Clinical Research and Advanced Medical Technology at Kumamoto University. Blood samples were collected from 7 HIV1/HTLV-1 coinfecting patients (Table 1), 12 HIV-1 mono-infected patients (Supplementary Table 1) and 13 HTLV-1 mono-infected patients. Peripheral blood mononuclear cells (PBMCs) were isolated using

Histopaque-1077 (Sigma-Aldrich) and cryopreserved in FBS (Gibco) containing 10% DMSO (Sigma-Aldrich). DNA extraction was carried out using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol.

Proviral load (PVL)

We estimated the PVL of the respective virus in PBMCs by quantifying the copy number of the *gag* gene for HIV-1 and the *tax* gene for HTLV-1, normalized to the copy number of the *ALB* gene, using real-time PCR, as previously reported [23] [24]. A complete description of the reaction conditions, and primer sequences is given in supplementary methods.

Integration site analysis by high throughput method

Integration site analysis of HIV-1 and HTLV-1 was performed using linker-mediated PCR (LM-PCR) and high-throughput sequencing as previously described [7] [25]. Three μg of genomic DNA were fragmented by sonication with a Picoruptor device (Diagenode, S.A., Belgium) to produce fragments in the range of 300–500bp. The DNA ends were repaired, and the DNA linkers were added. The junctions between the 3' LTR of HIV-1 or HTLV-1 and the host genomic DNA were amplified with the primers targeting the 3' LTR in the retroviruses and another targeting the linker. After a second PCR, amplicons were quantified using Illumina P5 and P7 primers. DNA libraries were sequenced by Illumina MiSeq as paired-end reads, and three fastq files, Read1, Read2, and Index Read were generated. The oligonucleotides used in LM-PCR and sequencing by Illumina MiSeq are listed in supplementary methods. The description of *in silico* analysis is also given in supplementary methods.

Statistical analysis

To quantify clonality, we calculated an oligoclonality index (OCI), which is based on the Gini Index using the total number of unique integration sites present in a particular person [7]. The Gini coefficient was calculated using the *reldist* R package. This index measures the non-uniformity of the distribution of clone abundance: a value of 0 indicates that all clones have the same abundance, and 1 implies that only a single clone is present. The OCI was calculated for samples with >5 unique

integration sites to limit underestimation of the OCI due to a small number of detected clones [26]. Statistical significance was analyzed by Prism 7 software (v7.04, GraphPad Software, Inc., CA).

Results

Quantification of PVL of HIV-1 and HTLV-1

We obtained the clinical data and the PBMCs of 2 time-points, between 1 and 6 years apart. Seven coinfecting individuals without malignant disease were enrolled in this study. Their clinical data are shown in Table 1. All blood samples were taken from coinfecting patients under treatment with cART, and in each case the HIV-1 RNA count in plasma was suppressed, except in two samples (3U and 3V at the earlier time-point) which were obtained from patients before the initiation of cART. Thus, the analysis included data from only 2nd time points in coinfecting patients to maintain consistency in study design.

Figure 1A shows CD4⁺ T-cell counts in patients with coinfection and HIV-1 mono-infection. The CD4⁺ T-cell count was not significantly different between coinfecting and HIV-1 mono-infected patients (median, 910.0/ μ l and 624.5/ μ l; Figure 1A). The HIV-1 PVL in coinfecting patients was significantly higher than that in HIV-1 mono-infected ones (median PVL = 4.0 and 1.1 copies/10,000 PBMCs respectively; Figure 1B). The same tendency was also observed in the HTLV-1 PVL (median PVL = 914.0 and 50.0 copies/10,000 PBMCs in coinfecting and mono-infected patients respectively; Figure 1B). The PVL in coinfecting patients was clearly different between HIV-1 and HTLV-1 (median PVL = 4.0 and 914.0 copies/10,000 PBMCs respectively, $p < 0.001$; Figure 1B).

Clonality of HIV-1 and HTLV-1-infected cells

We evaluated the clonality of HIV-1- and HTLV-1-infected cells in each individual by high-throughput integration site analysis (Figure 2, Supplementary Table 2). Because there were few detectable copies of HIV-1 in two patients (3Y and 3N), the data from these patients were excluded in the following clonality analysis in HIV-1. As shown in Figure 2, the number of detectable clones in HTLV-1 was greater than that of HIV-1. Some HIV-1-infected clones were expanded in each patient (Figure 2), however, the clonal abundance of each HIV-1-infected cell was much smaller than HTLV-1 due to the significantly smaller total number of HIV-1-infected cells (Figure 1B and 2B). The ten largest infected clones are coloured in each virus and each individual. The same colour represents a clone detected at both earlier and later time-points. The expanded clones with HTLV-1 were repeatedly detected in each patient at successive time-points, while few identical clones with HIV-1 were detected more than once. To compare the proportion of repeatedly detected clones between the two viruses, we quantified the detected clones of each virus in both 1st and 2nd time points. There was no difference between HIV-1 and HTLV-1 in the proportion of repeatedly detected clones [25 of 998 (2.5%) and 147 of 6909 (2.2%) respectively].

We next compared the degree of clonal proliferation of infected cells between mono-infection and coinfection. The clonal abundance of HIV-1 tended to be higher than that in mono-infection ($p = 0.05$; Figure 3A). Also, the clonal abundance of HTLV-1 was greater in coinfection than in mono-infection ($p < 0.001$; Figure 3A). Furthermore, we calculated the OCI, which quantifies the degree of clonal expansion of retrovirus-infected cells (see Methods for more details), for HIV-1- and HTLV-1-infected clones. Because the numbers of HIV-1 detected clones in 3N and 3Y were less than five, these two data were excluded from OCI analysis to accurately determine OCI. The OCI of HIV-1 in coinfection was significantly higher than that in mono-infection (median OCI = 0.249 and 0.038, respectively; $p < 0.01$; Figure 3B). Similarly, the OCI of HTLV-1 was greater in coinfection than in mono-infection (median OCI = 0.497 and 0.249, respectively; $p < 0.01$; Figure 3B). These

data show that HIV-1/HTLV-1 coinfection increases the oligoclonality of both HIV-1-infected and HTLV-1-infected cells.

The genomic environment of integrated HIV-1 and HTLV-1

Integration site analysis by the high-throughput method has revealed that HIV-1 and HTLV-1 preferentially target certain host genomic environments *in vivo*, specific features of which are associated with the abundance of the virus-infected clones [8, 9, 14]. We compared the genomic environments of integrated HIV-1 and HTLV-1 in the coinfecting patients with those in HIV-1 and HTLV-1 mono-infected ones. The majority of HIV-1 integration sites in both coinfection and mono-infection were located within introns in genic regions, as in previous reports (Figure 4A). The proportion of HIV-1 integration sites within genic regions was significantly lower in coinfecting patients (55%) than in HIV-1 mono-infected ones (70%).

Because the previous reports revealed that HIV-1 integration into cancer-related genes contributes to the clonal expansion under treatment with cART [11, 12], we performed GO enrichment analysis on HIV-1 integration sites in both coinfection and mono-infection. The HIV-1 integration sites in coinfecting subjects were enriched in genes related to lymphocyte differentiation and T-cell activation, while those in HIV-1 mono-infected cases were enriched in genes related to nuclear transport, RNA splicing, and T-cell receptor (Supplementary Figure 1). However, analysis of HIV-1 IS in expanded clones of co-infected individuals did not reveal enrichment of any specific ontological category, suggesting that integration into genes with specific gene ontology was not a major driver of clonal expansion of HIV-1-infected cells in this study.

Regarding HTLV-1, no significant difference between coinfecting and mono-infected patients was found in the proportion of integration sites in genic regions: 39% in mono-infection and 42% in coinfection. Among viruses integrated into genic regions, the orientation of each viral transcription relative to host genes did not differ between coinfecting and mono-infected patients (Figure 4B).

We further investigated whether coinfection altered the epigenetic characteristics associated with the HIV-1 or HTLV-1 integration sites. First, we analyzed frequencies of viral integration sites within ± 2 kb of histone marks compared to random expectation. In line with previous reports, HIV-1 integration sites, especially in mono-infection, were enriched in regions with activating histone marks, including H3K27ac, H3K4me3, H3Kme1, and H3Kme36me3; but not in those with repressive histone marks, such as H3K27me3 and H3K9me3 (Figure 4C). HTLV-1 integration in both coinfecting and mono-infected patients was significantly more frequent than random expectation in regions with H3K27ac and H3K4me3 marks, associated respectively with active transcription and gene promoters, and with H3K9me3, which is a mark of constitutive heterochromatin (Figure 4D). To compare the frequencies of integration sites near each histone mark between coinfection and mono-infection, we calculated an odds ratio. HIV-1 integration in coinfection was more frequently detected near repressive histone marks, H3K27me3 and H3K9me3, compared to mono-infection, while HTLV-1 integration in coinfection was less frequent near H3K9me3 compared to mono-infection (Figure 4D).

Discussion

cART can strongly inhibit HIV-1 replication and prevent the development of AIDS. However, HIV-1 persists at low levels as a provirus in the host cellular genomic DNA, even under cART. Recent studies revealed that some HIV-1-infected cells are also clonally expanded and play a role in viral persistence *in vivo* [8, 9, 27]. Since both HIV-1 and HTLV-1 infect CD4⁺ T-cells, coinfection could affect the clonal expansion of HIV-1- or HTLV-1-infected cells. In this study, we demonstrated that the PVL and OCI of both HIV-1 and HTLV-1 in coinfecting patients were higher than those in the respective mono-infected patients. The high OCI indicates that virus-infected cells in coinfection exhibit a higher degree of selective clonal expansion than those in mono-infection.

Previous studies revealed that HIV-1 integration is enriched in highly expressed genes, and clonally expanded clones of HIV-1 increase over time under cART [27]. HIV-1 integration into cancer-related genes contributes to the clonal expansion in persistent infection [8, 9]. In this study, we

showed the HIV-1 integration sites in coinfection tended to be enriched in transcriptionally repressive regions when compared to mono-infection, despite the higher degree of clonal expansion observed in coinfection. The clonal expansion of HIV-1-infected cells in coinfection was not associated with integration into cancer-related genes (Supplementary Figure 1), however, this conclusion requires corroboration in further studies. It is unclear how HTLV-1 infection is involved in the clonal expansion of HIV-1-infected cells in coinfecting patients. One possible explanation is that soluble factors produced by HTLV-1-infected cells are capable of enhancing the proliferation of HIV-1-infected cells. The proliferation of HTLV-1-infected cells is known to be supported by their dependence on several cytokines, such as IL-2, IL-4, IL-6, and IL-13. HTLV-1-infected cells express high levels of the IL-2 receptor (CD25) due to the transcriptional effect of Tax on the CD25 promoter, and the soluble form of IL-2 receptor is a sensitive prognostic marker in ATL [28] [29]. Interestingly, it has been reported that latent HIV-1-infected CD4⁺ T-cells can proliferate in response to cytokines such as IL-2 and IL-7 without viral reactivation [30], suggesting that secretion of IL-2 may contribute to clonal expansion of HIV-1-infected cells in coinfecting patients. The other potential cause is antigen-driven clonal selection in HIV-1 persistent. Simonetti FR. et al. showed that it is possible for proliferation of HIV-1-infected cells to occur under cytomegalovirus-antigenic stimulation in individuals on cART [11]. The proliferation of infected cells under antigenic stimulation was observed regardless of the integration site. In HIV-1/HTLV-1 coinfecting individuals, one virus-antigen stimulation might influence the clonal expansion of other virus-infected cells. Attention should be paid to the fact that, the duration of cART in coinfecting individuals was shorter than that of HIV-1 mono-infected ones (median duration times, 7 and more than 10 years, respectively). Previous studies reported that clonally expanded cells in HIV-1-infected individuals on cART increase over time [8] [9]. It is possible that the duration of cART may be associated with different degrees of clonality of HIV-1-infected cells between coinfection and HIV-1 mono-infection.

We also observed one virus affecting the integration sites of the other in coinfection as well as the degree of clonal expansion. One potential explanation is that HIV-1 was integrated into a cell also containing an HTLV-1 provirus. Thus, the increase of HIV-1-infected cells could result from the

clonal expansion of CD4⁺ T-cells infected with HTLV-1. To analyze whether both HIV-1 and HTLV-1 are present in the same cell, we analyzed the frequency of HIV-1 infection in HTLV-1 infected cells (data not shown). We observed dual infection of HIV-1 and HTLV-1 in the same cell, but it is extremely rare. Josefsson et al. reported that the majority of naturally HIV-1-infected clones carry a single provirus in HIV-1-infected individuals [31]. Cook et al. also showed the evidence that a single HTLV-1 provirus was present in every infected clone in non-malignant HTLV-1-infected individuals [32], though it is known that ATL clones often carry multiple integrated HTLV-1 proviruses [33]. Multiple proviral integration in a single cell can occur, but is uncommon.

The greater PVL and OCI in coinfection were also observed with not only HIV-1 infection but also HTLV-1 infection. The cohort of HTLV-1 mono-infected patients in the present study were asymptomatic carriers and presented a low PVL (Figure 1B). The larger cohort study showed the median PVL of asymptomatic HTLV-1 carriers was 160.0 copies/10,000 PBMCs (range 0-5,580/10,000 PBMCs) [34]. The coinfecting patients in the present study had a significantly higher PVL than those in the larger cohort (median PVL = 914.0 and 160.0 copies/10,000 PBMCs, respectively). The efficiency of the host's immune response to HTLV-1, especially the HTLV-1-specific CTLs, plays an important role in determining the total number of HTLV-1-infected clones [35]. Remarkably, even in the context of cART-induced viral suppression, CD8⁺ T-cells dysfunction is present in HIV-1-infected individuals. CD8⁺ T-cells from HIV-infected individuals exhibit increased expression of inhibitory receptors such as programmed cell death protein 1 (PD-1), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte Activation Gene-3 (LAG-3), CD160, and 2B4 [36] [37] [38]. These receptors interfere with TCR signaling, resulting in decreased response of antigen-specific cells. Besides, although CD8⁺ T-cell counts are elevated in HIV-1-infected individuals treated with cART [39], the population of CD8⁺ T-cells with memory subsets is not fully reconstituted [40]. These data indicate that coinfecting patients could be less capable of restricting clone abundance than those with HTLV-1 mono-infection.

The genomic environments of integrated HIV-1 and HTLV-1 in coinfection were similar to those in each viral mono-infection (Figure 4C). When we quantified the odds ratios of integration

sites near histone marks between coinfection and mono-infection, HIV-1 integration in coinfection had a bias towards sites rich in repressive histone marks (Figure 4D). There are two possible explanations for this finding: either HIV-1/HTLV-1 coinfection impacts HIV-1 integration to transcriptionally silent regions of the host genome, or active selection during latent infection favours clones with HIV-1 integrated in the regions with repressive histone marks. Cohn LB. et al. reported that the surviving HIV-1-infected cells under cART were enriched in silent regions of the genome [41], supporting the latter potential explanation.

HIV and HTLV insertional mutagenesis have been previously thought to potentially contribute the pathogenesis of clonal expansion of their infected cells, respectively. Liu R. et al showed that HIV-1 drives high aberrant host gene transcription downstream of the integration site through HIV-1-host aberrant splicing [42]. Also, HIV-1 integrated into the *STAT3* gene generates hybrid transcripts splicing HIV to *STAT3* sequences, supporting a model of LTR-driven *STAT3* overexpression as a driver of preferential growth [10]. In terms of HTLV-1, the provirus forms reproducible abnormal chromatin contacts with sites in the host genome in cis, and some of these abnormal chromatin contacts depend on CTCF binding to the provirus. [43, 44]. These results imply that both viruses have the potential to cause dysregulation of host transcription in each infected host.

There are several limitations in this study. The PVL were not detected in three HTLV-1 mono-infected individuals. The lower limit of quantification has been reported to be between 10^{-5} and 10^{-4} [45, 46]. Another possible cause is sequence variation in the primer-binding region of the provirus. In our IS analysis by high throughput method, this factor might also preclude detection of certain clones (Figure 2). The fact that we may miss some clones in the IS analysis must be taken into consideration when interpreting the clonality data. Several alternative methods for IS analysis to overcome this point have been developed [47] [48].

In summary, we performed a comprehensive analysis of the integration sites and evaluated the clonality of infected cells in patients with HIV-1/HTLV-1 coinfection. By comparison with HIV-1 or HTLV-1 mono-infected individuals, a higher degree of PVL and clonal expansion of both HIV-1- and HTLV-1-infected cells were observed in coinfection. These findings suggest that coinfection might lead to a different clinical outcome compared with mono-infections. Larger longitudinal studies are warranted to reveal pathogenesis and appropriate treatment strategies for patients coinfecting with these two viruses.

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Conflict of Interests:

All authors declare no competing interests.

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Figure legends

Figure 1. The number of CD4⁺ T-cells and proviral load.

A, the number of CD4⁺ T-cells was measured during treatment with cART for HIV-1 mono-infected and HIV-1/HTLV-1 coinfecting patients. B, proviral load (PVL) indicates the number of virus-infected cells per 10,000 peripheral blood mononuclear cells. Each HIV-1 and HTLV-1 PVL in mono-infected and coinfecting patients was measured by real-time PCR. The analysis included data at 2nd time point of 7 coinfecting individuals and all mono-infected ones. The bars indicate median values with 95% confidence interval. Statistical significance was obtained by Mann-Whitney U test (* <0.05, ** <0.01, *** <0.001). n.s, not significant.

Figure 2. Clonality of HIV-1- and HTLV-1-infected cells in coinfection.

The distribution of HIV-1- and HTLV-1-infected clones in the coinfecting patients. Each sector in the pie-charts depicts the relative abundance of the respective integration site. The ten largest infected clones between the two time-points are coloured in each individual. The same colour denotes the clones detected at both earlier and later time-points. The same colour between HIV-1 and HTLV-1 does not correspond to the same clone. The number of detectable unique clones is shown under each pie-chart.

Figure 3. Comparison of clonal abundance between mono-infection and coinfection.

A, Degrees of abundance of each individual clone are shown in the scatter dot plot. The analysis included data at 2nd time point of 7 coinfecting individuals and all mono-infected ones. B, The oligoclonality index (OCI) is a measure of the non-uniformity of the frequency distribution. A value of 0 indicates that all clones have the same abundance and 1 implies that only a single clone is present. Because the numbers of HIV-1 detected clones in 3N and 3Y were less than five, these two

data were excluded from OCI analysis to accurately determine OCI. The bars indicate median values with 95% confidence interval. Statistical significance was obtained by Mann-Whitney U test (* <0.05, ** < 0.01, *** <0.001). n.s, not significant.

Figure 4. The genomic environments flanking HIV-1 and HTLV-1 proviruses

A, Proportion of viral integration sites in genic (intron and exon), and intergenic regions. This analysis included integration sites at 2nd time point of 7 coinfecting individuals and all mono-infected ones. The numbers of integration sites are shown at the top of each bar graph. B, Proportion of the orientation of each viral transcription relative to host genes. The orientation of +/- indicates that an integration site present in the regions encoded by several genes with opposite direction. The numbers of integration sites within genes included in the analysis are shown at the top of each bar graph. C, Integration frequencies near the activating histone marks including H3K27ac, H3K4me3, H3Kme1, and H3Kme36me3, and the repressive histone marks with H3K27me3 and H3K9me3. Integration sites within ±2kb of each histone mark were compared to random expected values. Fold enrichment is represented as the ratio of observed sites/random expected sites. D, The odds ratio of viral integration sites within ±2kb of each histone mark, comparing coinfection and mono-infection. The analysis of C and D included integration sites at 2nd time point of 7 coinfecting individuals and all mono-infected ones.

Statistical significance was assessed by Fisher's test in A and B (* <0.05, *** <0.001).

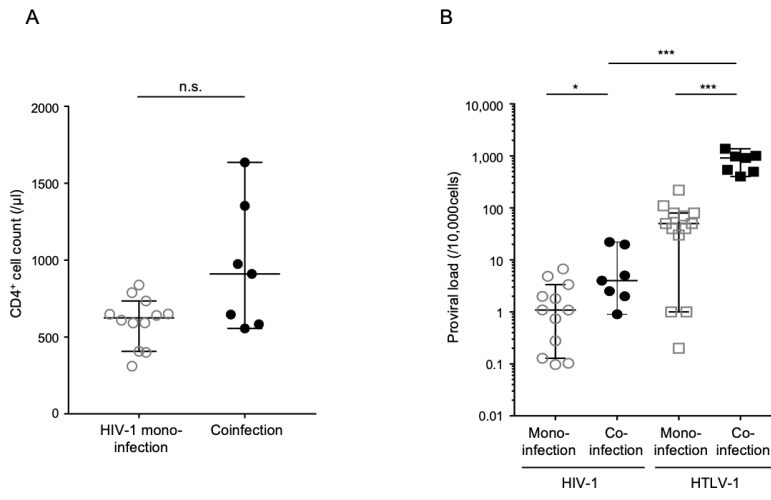
The q values are measured using Storey and Tibshirani's False Discovery Rate for multiple testing correction in C and D (* <0.05, ** <0.01, *** <0.001).

Table 1. Characteristics of HIV-1/HTLV-1 coinfecting patients

Pt.	1st time point						2nd time point					
	age	CD4 ⁺ count (/μl)	Plasma HIV (copies/ml)	PVL (/10,000cells)		cART	age	CD4 ⁺ count (/μl)	Plasma HIV (copies/ml)	PVL (/10,000cells)		cART
				HTLV -1	HIV -1					HTLV -1	HIV -1	
3TD J	59	600	<50	1699	5	FPV/r, ZDV, ABC	65	910	<20	914	2	FPV/r, ZDV, ABC
3P	71	582	<50	962	0.3	LPV/r, TDF, FTC	76	583	<20	497	0.9	ATV/r, ABC, 3TC
3Q	51	806	<50	429	5.4	NVP, TDF, 3TC	55	975	<20	401	2.5	RAL, NVP, 3TC
3Y	37	na	40	560	19.2	RPV, TDF, FTC	38	646	<20	1370	19.7	RPV, TDF, FTC
3N	44	839	84	760	17.7	LPV/r, TDF, FTC	45	1636	<20	1000	22.1	LPV/r, TDF, FTC
3U	52	115	41,649	520	4	before ART	55	556	na	542	5	DRV/r, TDF, FTC
3V	51	2163	7,000,000	4290	99	before ART	54	1353	<40	972	4	EFV, ZDV, 3TC

PVL, proviral load; FPV/r, fosamprenavir/ritonavir; ZDV, zidovudine; ABC, abacavir; LPV/r, lopinavir/ritonavir; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; NVP, Nevirapine; 3TC, lamivudine; ATV/r, atazanavir/ritonavir; RAL, raltegravir; DRV/r, darunavir/ritonavir; EFV, efavirenz; RPV, rilpivirine; na, not available

Figure 1



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Figure 2

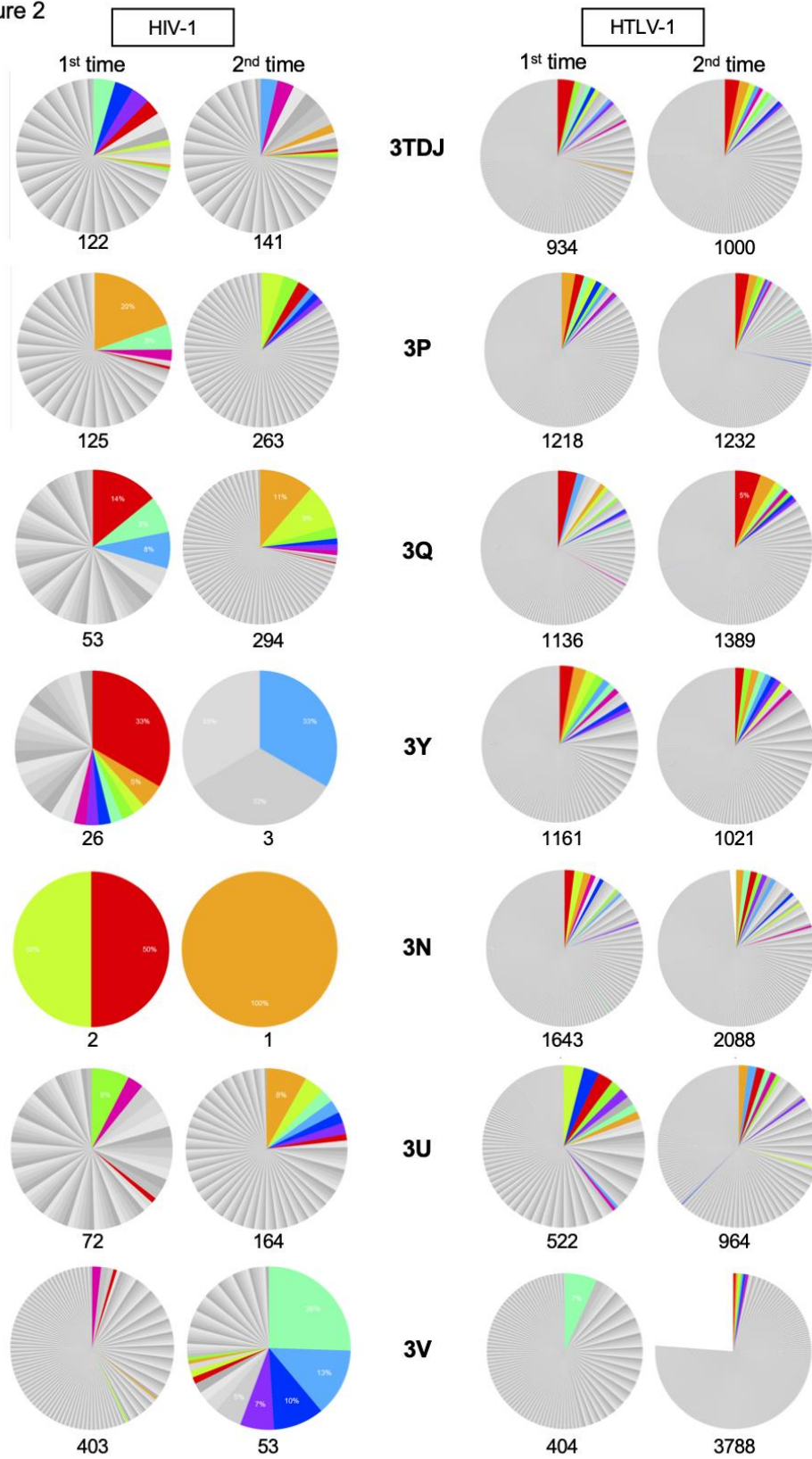
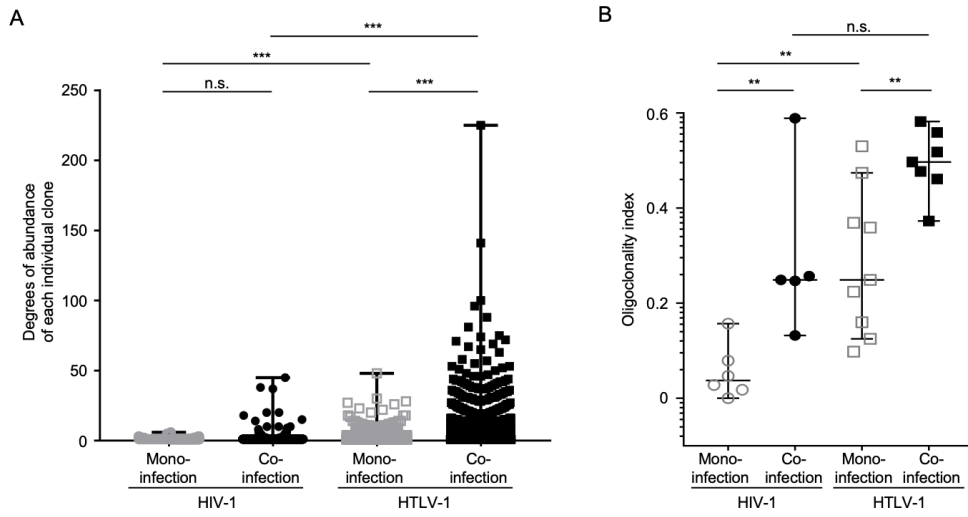


Figure 3



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Figure 4

