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MAJOR ARTICLE

Naive CD4+ T Cells Harbor a Large Inducible Reservoir of Latent, Replication-competent Human Immunodeficiency Virus Type 1

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Background. The latent human immunodeficiency virus type 1 (HIV-1) reservoir represents a major barrier to a cure. Based on the levels of HIV-1 DNA in naive (T_N) vs resting memory CD4+ T cells, it is widely hypothesized that this reservoir resides primarily within memory cells. Here, we compared virus production from T_N and central memory (T_{CM}) CD4+ T cells isolated from HIV-1–infected individuals on suppressive therapy.

Methods. CD4+ T_N and T_{CM} cells were purified from the blood of 7 HIV-1–infected individuals. We quantified total HIV-1 DNA in the CD4+ T_N and TCM cells. Extracellular virion-associated HIV-1 RNA or viral outgrowth assays were used to assess latency reversal following treatment with anti-CD3/CD28 monoclonal antibodies (mAbs), phytohaemagglutinin/interleukin-2, phorbol 12-myristate 13-acetate/ionomycin, prostratin, panobinostat, or romidepsin.

Results. HIV-1 DNA was significantly higher in T_{CM} compared to T_N cells (2179 vs 684 copies/10^6 cells, respectively). Following exposure to anti-CD3/CD28 mAbs, virion-associated HIV-1 RNA levels were similar between T_{CM} and T_N cells (15 135 vs 18 290 copies/mL, respectively). In 4/7 donors, virus production was higher for T_N cells independent of the latency reversing agent used. Replication-competent virus was recovered from both T_N and T_{CM} cells.

Conclusions. Although the frequency of HIV-1 infection is lower in T_N compared to T_{CM} cells, as much virus is produced from the T_N population after latency reversal. This finding suggests that quantifying HIV-1 DNA alone may not predict the size of the inducible latent reservoir and that T_N cells may be an important reservoir of latent HIV-1.

Keywords. HIV-1; latent; reservoir; naive; memory.

The reservoir of latent human immunodeficiency virus type 1 (HIV-1) that resides in resting CD4+ T cells constitutes a major barrier to a cure [1–5]. This reservoir is unaffected by the immune system or by antiretroviral therapy (ART) but has the potential to produce infectious virus, which may contribute to persistent plasma viremia during ART or viral rebound if ART is interrupted. The resting CD4+ T-cell population, however, is heterogeneous, and HIV-1 DNA has been detected in a number of different T-cell subsets, including naive (T_N), stem cell-like memory (T_{SCM}), central memory (T_{CM}), transitional memory (T_{TM}), effector memory (T_{EM}), and terminally differentiated (T_{TD}) cells [6–13]. Several studies have tried to define the relative contributions of each of these subsets to the total pool of latent HIV-1 infection by comparing total viral DNA in the sorted CD4+ T-cell subsets in peripheral blood [6–8, 12]. In general, these studies found that the T_{CM}, T_{TM}, and T_{EM} cell populations consistently contained higher levels of HIV-1 DNA than T_N cells. Consequently, reservoir studies have primarily focused on the memory CD4+ T-cell compartment, and the T_N cell population has been largely overlooked.

In our laboratory, we recently used a primary cell model of HIV-1 latency to address differences in the establishment and reversal of viral latency in highly purified T_N and T_{CM} cells [14]. Consistent with previously published studies [15–18], we found that HIV-1 infected T_N cells less efficiently than T_{CM} cells. However, when the infected T_N cells were treated with latency reversing agents (LRAs), including anti-CD3/CD28 monoclonal antibodies (mAbs), phorbol-myristate-acetate (PMA)/phytohaemagglutinin (PHA), and prostratin, as much, if not more, extracellular virion-associated HIV-1 RNA was produced per infected T_N cell compared to infected T_{CM} cell. There were no major differences in the genomic distribution of HIV-1 integration sites between T_N and T_{CM} cells that accounted for these observed differences [14]. These findings suggested that T_N cells may be an important reservoir of latent HIV-1 infection and should not be overlooked simply because the frequency of infection of these cells is lower than in the memory T-cell subsets. To validate these in vitro findings,

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in this study, we quantified the inducible latent HIV-1 reservoirs in CD4+ T_N and T_{CM} cells from infected individuals on suppressive ART.

METHODS

Isolation of CD4+ T_N and T_{CM} Cells From HIV-1–infected Individuals on ART

Leukapheresis was performed on 7 HIV-1–infected donors who were on suppressive ART (<20 copies of HIV-1 RNA/mL plasma) for ≥5 years (Table 1). We obtained approximately 2×10^9 peripheral blood mononuclear cells (PBMCs) from each leukapheresis to complete the studies described. All donors provided written informed consent, and the University of Pittsburgh Institutional Review Board approved the blood donation protocol. CD4+ T_N cells were isolated as previously described [14]. In 3 donors, the T_{SCM} cells were removed from the T_N population by depletion of CD95+ cells. T_{CM} cells were isolated as previously described [14], with the addition of positive selection for CD62L+ cells after isolating the CD45RA- cells. The CD62L- fraction was also saved as the T_{TM}+T_{EM} cell population. In Supplementary Figure 1, we provide a detailed schematic that outlines our purification strategy. All magnetic bead purification kits and antibodies were from Miltenyi Biotec (Auburn, CA). The purity of the T_N and T_{CM} cells was assessed by flow cytometry (LSR II, BD Biosciences) using the following antibodies: CD3-V450, CD4-PerCP-Cy5.5, CD45RA-FITC, CCR7-PE, CD27-APC-H7, and CD62L-APC (BD Biosciences). Data were analyzed using FlowJo vX.0.7. The T_N and T_{CM} cell surface phenotypes were as follows: T_N cells (CD45RA+, CCR7+, CD27+, CD62L+, [CD95-]) and T_{CM} cells (CD45RA-, CCR7+, CD27+, CD62L+). T_N and T_{CM} cell purity was routinely found to be ≥98 % and ≥96 %, respectively (Supplementary Figure 2).

Ex vivo Reactivation of Latent HIV-1 From CD4+ T_N and T_{CM} Cells

Purified T_N and T_{CM} cells were plated in 24-well plates at a density of 10^6 cells/well and cultured in the presence of 300 nM efavirenz and 300 nM raltegravir to prevent viral spread. Cells were activated for 7 days in duplicate wells with anti-CD3/CD28 mAbs (3 beads per cell; Life Technologies), 10 μg/mL PHA (Remel) + 100 U/mL interleukin 2 (IL-2; Roche), 5 nM PMA + 500 μg/mL ionomycin (Sigma), 5 μM prostratin, 17 nM panobinostat (Selleckchem; pulsed for 30 minutes), or 50 nM romidepsin (Selleckchem; pulsed for 4 hours). Unstimulated cells were used as a negative control. Two and 4 days post-activation, IL-2 (10U/mL), efavirenz (300nM), and raltegravir (300nM) were added to each well.

Quantification of Total HIV-1 DNA

Total HIV-1 DNA was quantified in freshly isolated T-cell subsets as defined in Supplementary Figure 3, as well as in pooled duplicate culture wells. Total cellular DNA was extracted and was assayed for total HIV-1 DNA by quantitative polymerase chain reaction assay (PCR), as described previously [19, 20]. Each sample was run in triplicate using the LightCycler 480 System (Roche). DNA standards were included, as described previously [21]. HIV-1 DNA was normalized to the total number of cells assayed by quantitative PCR amplification of the *CCR5* gene [22].

Quantification of Extracellular Virion-associated HIV-1 RNA

Extracellular virion-associated HIV-1 RNA was extracted and quantified as previously described [14].

Quantitative Viral Outgrowth Assay

The quantitative viral outgrowth assay was carried out as previously described [23]. Infectious units per million cells (IUPM) were calculated as described previously [23, 24]. Outgrowth positive wells were determined using a p24 enzyme-linked immunosorbent assay (ZeptoMetrix Corporation).

Flow Cytometry

T-cell activation was assessed by flow cytometry using the following antibodies (from BD Biosciences): CD3-V450, CD4-PerCP-Cy5.5, CD25-PE-Cy7, CD69-PE, and HLA-DR-FITC. Cell viability was determined using a LIVE/DEAD fixable cell viability dye for flow cytometry (Invitrogen). All samples were run on an LSRII, and the data were analyzed using FlowJo vX.0.7.

Table 1. Characteristics of Study Participants

Donor No.	Sex	Age (y)	Race	Current CD4 Count, cells/mm^3	Pre-ART Viral Load, copies of HIV-1 RNA/mL	Years of Suppression	Current ART Regimen
1	F	36	Black	809	802 000	10.2	TDF/FTC/RPV
2	F	57	Black	380	143 000	5.2	TDF/FTC/ATZ/r
3	M	57	White	656	520 000	12	TDF/FTC/EFV
4	M	51	White	603	Unknown	7.3	TAF/FTC/EVG/c
5	M	46	Black	714	41 952	6.0	ABC/3TC/EFV
6	F	58	Black	1426	366 200	15	TDF/FTC/RPV
7	F	62	Black	1033	Unknown	10.7	TDF/FTC/DTG
Mean		52		803		9.5	

Abbreviations: 3TC, lamivudine; ABC, abacavir; ATZ/r, atazanavir/ritonavir; DTG, dolutegravir; EFV, efavirenz; EVG/c, elvitegravir/cobicistat; FTC, emtricitabine; RPV, riplivirine; TAF, tenofovir alafenamide; TDF, tenofovir disproxil fumarate.

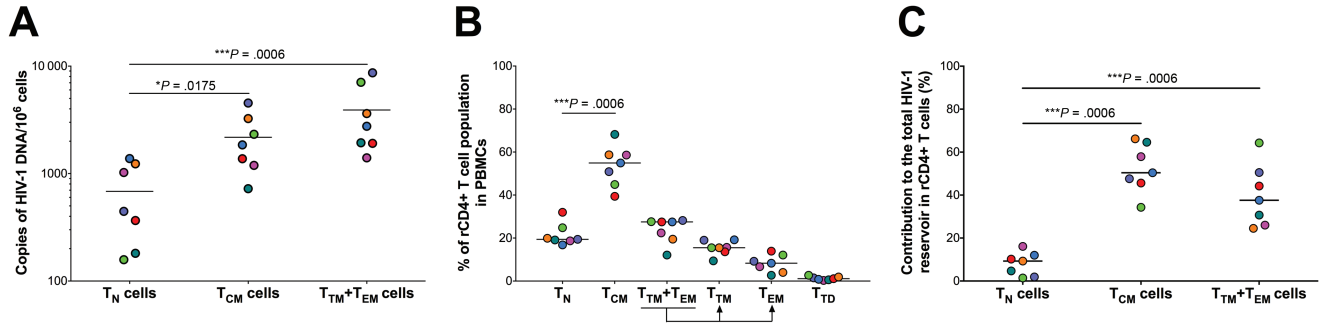

Figure 1. Quantification of the total human immunodeficiency virus type 1 (HIV-1) DNA reservoir in CD4+ T naive (T_N), T central memory (T_{CM}), transitional memory (T_{TM})+effector memory (T_{EM}) cells purified from HIV-infected individuals on antiretroviral therapy. *A*, Quantification of total HIV-1 DNA in CD4+ T_N, T_{CM}, and T_{TM}+T_{EM} cells. Total HIV-1 DNA was normalized to the cell number (determined by quantification of the *CCR5* gene). *B*, The frequency of different resting CD4 T-cell subsets in the peripheral blood of HIV-1–infected individuals. *C*, Contribution (%) of the CD4+ T_N, T_{CM}, and T_{TM}+T_{EM} cells to the total HIV-1 DNA reservoir, taking into account both the frequency of the CD4+ T-cell subset and the frequency of HIV-1 DNA in that subset. In the plots, each colored dot represents a unique donor. All *P* values were determined using a Mann–Whitney test. Abbreviation: PBMC, peripheral blood mononuclear cell.

Statistical Analyses

Statistical comparison between paired samples was performed using a Wilcoxon matched-pairs signed rank test. For all unpaired samples, statistics were determined using a Mann-Whitney test. For statistical comparisons between unpaired samples where N <6, statistics were determined using an unpaired *t* test. For all statistical analyses, $P < .05$ was considered significant. All statistics were calculated in GraphPad Prism v6.0.

RESULTS

Donor Characteristics

Experiments were performed using PBMC obtained from 7 (4 females, 3 males) chronically HIV-1–infected donors on suppressive ART who met the eligibility criterion of having plasma HIV-1 RNA ≤20 copies/mL for ≥5 years, with a median of 9.5 years (Table 1). The median age was 52 years. Five of the donors were black and 2 were white. The median CD4+ T-cell count at the time of leukapheresis was 803 cells/mm^3.

CD4+ T_N Cells Harbor Less Total HIV-1 DNA Than T_{CM} Cells

HIV-1 DNA was detectable in both the T_N and T_{CM} subsets in all 7 donors (Figure 1A, Supplementary Table 1). However, consistent with prior studies [6–8, 25], the levels of total HIV-1 DNA were significantly higher (median fold change, 5.4; range, 1.2–14.7; $P = .0175$) in the T_{CM} cells (mean, 2179 copies/10^6 cells; range, 723–4533) compared to T_N cells (mean, 684 copies/10^6 cells; range, 158–1380). We also quantified total HIV-1 DNA in the combined CD4+ T_{TM}/T_{EM} cell population (Figure 1A). These cells harbored slightly higher levels of HIV-1 DNA compared to the T_{CM} cells (mean fold change, 1.8; range, 1.1–3.0); however, this increase was not statistically significant. The T_{TM}/T_{EM} cell population, however, harbored significantly higher levels of total HIV-1 DNA

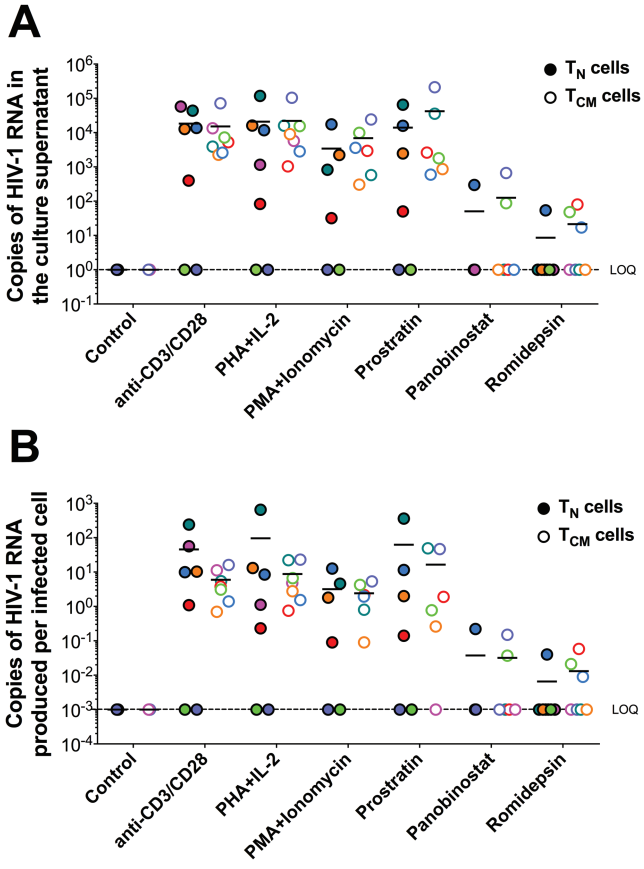

Figure 2. Total virus recovery from CD4+ T naive (T_N) and T central memory (T_{CM}) cells from human immunodeficiency virus type 1 (HIV-1)–infected individuals on antiretroviral therapy following latency reversal. *A*, Total copies of extracellular virion-associated HIV-1 RNA produced from T_N or T_{CM} cells after exposure to the latency reversing agents indicated. *B*, Copies of HIV-1 RNA produced per infected CD4+ T_N or T_{CM} cell. Each colored dot represents a unique donor. Solid dots represent data from CD4+ T_N cells, while open dots represent data from CD4+ T_{CM} cells. N = 7; except for phorbol-myristate-acetate +Iono, prostratin, and panobinostat, where N = 6. Abbreviations: HIV-1, human immunodeficiency virus type 1; Iono, ionomycin; LOQ, limit of quantification; PHA, phytohaemagglutinin; PMA, phorbol-myristate-acetate; T_{EM}, T effector memory cell.

vs the T_N cells ($P = .0006$). Next, we determined the contribution of each T-cell subset to the total HIV-1 reservoir in resting CD4+ T cells as previously described [12]. First, we estimated the frequency of each T-cell subset in the resting CD4+ T cells from each donor (Figure 1B, Supplementary Figure 3). We then calculated the contribution of each CD4+ T-cell subset to the overall reservoir of HIV-1 DNA in the resting CD4+ T-cell population by taking into consideration both the frequency of each T-cell subset in the peripheral blood, as well as the frequency of the total HIV-1 DNA in that subset. We found that the CD4+ T_{CM} population harbored the highest levels of total HIV-1 DNA (Figure 1C), consistent with previously published studies [12].

Similar Total Virus Recovery From CD4+ T_N and T_{CM} Cells Following Exposure to LRAs

We quantified total virus recovery (ie, extracellular virion-associated HIV-1 RNA in the culture supernatant) from the donor-derived T_N and T_{CM} cells after exposure to 6 different LRAs using an ultrasensitive assay capable of single-copy detection of HIV-1 RNA (Figure 2A, Supplementary Table 1). The mean number of copies of HIV-1 RNA produced per 10^6 cells was not significantly different between the T_N and T_{CM} subsets following treatment for any of the LRAs tested. Interestingly, in cells from 4 of the 7 donors (donors 1, 3, 5, and 7), the virion-associated HIV-1 RNA levels produced were higher for T_N cells compared to T_{CM} cells, independent of the

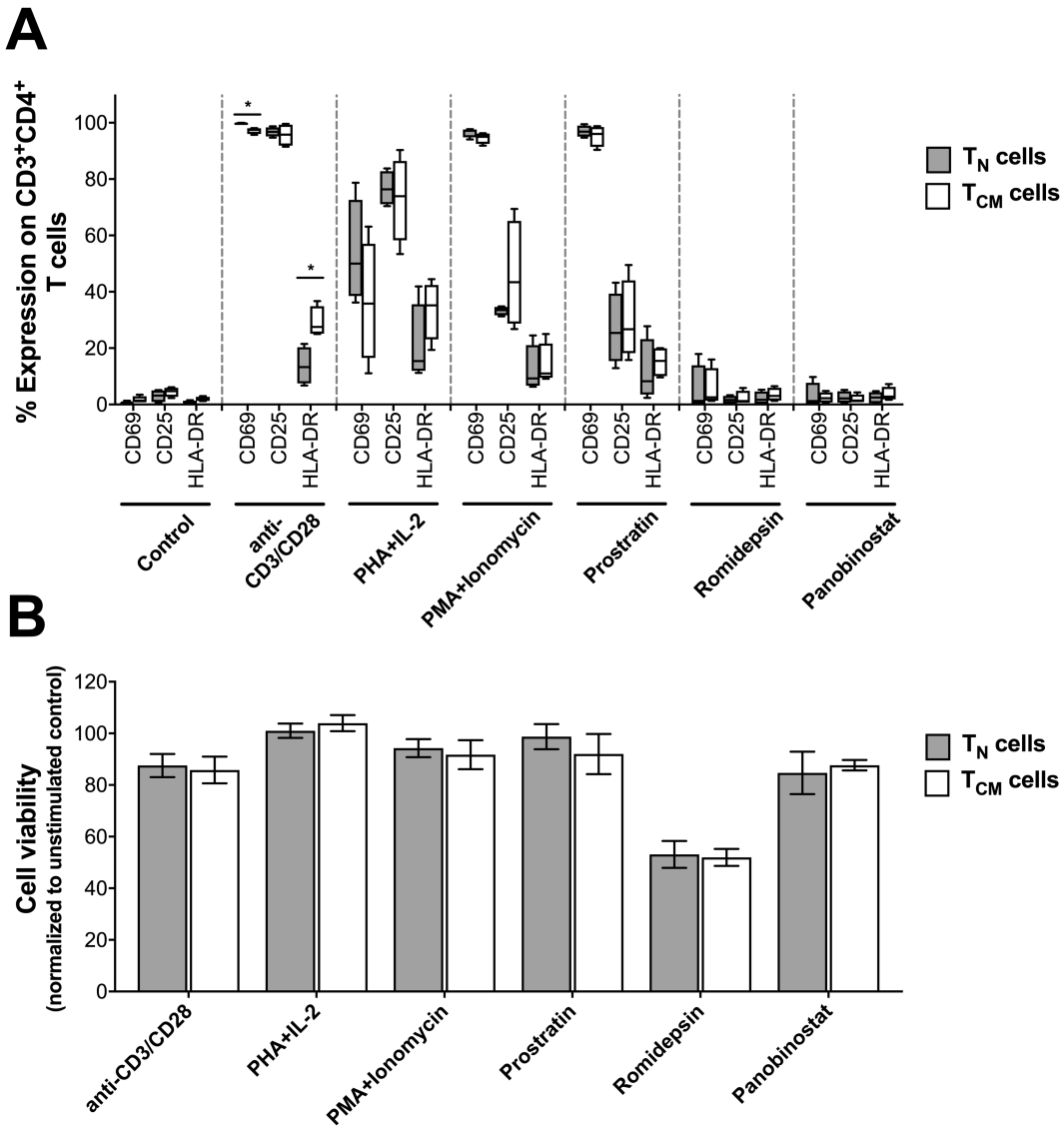

Figure 3. Impact of the latency reversing agent (LRA) on global T-cell activation and cell viability on CD4+ T naive (T_N) and T central memory (T_{CM}) cells purified from human immunodeficiency virus type 1 (HIV-1)–infected individuals on antiretroviral therapy. *A*, Expression of the T-cell activation markers CD25, CD69, and HLA-DR on CD4+ T_N and T_{CM} cells 7 days post-LRA exposure. All data are shown as the mean ± standard error of the mean (SEM; N = 7). *B*, CD4+ T_N or T_{CM} cell viability assessed 7 days post-LRA exposure. All data are shown as the mean ± SEM (N = 4). No significant differences were noted between the 2 cell types, as measured by a Mann–Whitney test. Abbreviations: Iono, ionomycin; PHA, phytohaemagglutinin; PMA, phorbol-myristate-acetate; T_{EM}, T effector memory cell.

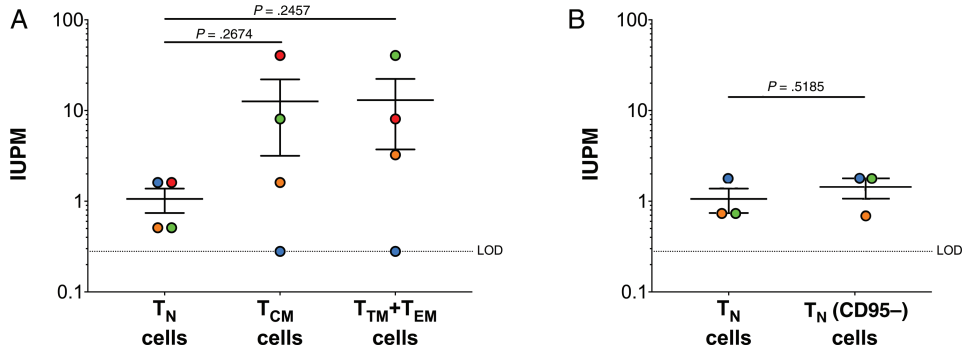

Figure 4. Quantification of replication-competent human immunodeficiency virus type 1 (HIV-1) in CD4+ T naive (T_N), T central memory (T_{CM}), transitional memory (T_{TM})+effector memory (T_{EM}) cells from HIV-1–infected donors on antiretroviral therapy. *A,* Infectious units per million cells (IUPM) values determined from T_N, T_{CM}, and T_{TM}+T_{EM} cells. *B,* IUPM values determined for T_N cells with (T_N cells) and without (T_NCD95-) T stem cell-like memory cells. Each colored dot represents a unique donor. Data are shown as the mean ± standard error of the mean. *P* values were determined using an unpaired *t* test.

LRA used (Supplementary Table 1). When the extracellular virion-associated RNA copies were normalized to the number of infected cells (Figure 2B), we found that more HIV-1 RNA was produced per infected T_N cell than T_{CM} cell when stimulated with anti-CD3/CD28 mAb, PHA+IL-2, PMA/PHA, or prostratin, although this did not reach statistical significance. In contrast, both romidepsin and panobinostat failed to increase virus production in either cell type in the majority of donors. This finding is consistent with other studies that demonstrated that histone deacetylase inhibitors do not consistently increase virus production ex vivo in resting CD4+ T cells from HIV-1–infected individuals on suppressive ART [26–31].

T-Cell Activation and Total Virus Recovery in CD4+ T_N and T_{CM} Cells

As described above, more HIV-1 RNA was produced per infected T_N cell compared to T_{CM} cell after exposure to anti-CD3/CD28 mAb, PHA+IL-2, PMA/PHA, or prostratin. Each of these LRAs induces T-cell activation. Therefore, we next assessed whether differences in T-cell activation between the T_N and T_{CM} cells, at the time of harvest, could potentially account for differences in total virus recovery. T-cell activation was assessed by measuring surface expression of CD25, CD69, and HLA-DR by flow cytometry (Figure 3A). We also evaluated cell viability by LIVE/DEAD staining (Figure 3B). As expected, anti-CD3/CD28 mAbs, PHA+IL-2, PMA+ionomycin, and prostratin induced T-cell activation in both T_N and T_{CM} cells. However, there were no significant differences between T_N and T_{CM} cells that could adequately account for the observed differences in total virus recovery (Figure 2A). Similarly, there were no significant differences in cell viability between T_N and T_{CM} cells following LRA stimulation (Figure 3B) or in controls (90.3% vs 89.5%, respectively).

Frequency of Replication-competent HIV-1 Is Similar in CD4+ T_N and T_{CM} Cells

The total virus recovery assay does not inform as to whether the HIV-1 is replication competent. Therefore, we also performed a quantitative viral outgrowth assay on a subset of the donors (donors 4–7) as previously described [23]. In these experiments, we included the T_{TM}+T_{EM} cell subset in addition to the T_N and T_{CM} cells. We detected replication-competent HIV-1 in the T_N cells from all 4 donors, but only 3 of the 4 donors in T_{CM} and T_{TM}+T_{EM} cells (Figure 4A). While on average higher IUPM values were determined in the T_{CM} and T_{TM}+T_{EM} cells compared to the T_N cells (Figure 4A), if we normalized these values to the number of infected cells, we found minimal differences in IUPM between the different subsets (Table 2).

Collectively, these data highlight that T_N cells are not only able to produce virus following latency reversal, but a portion of the virus produced from these cells is replication competent.

Table 2. Differences in Infectious Units per Million Cells and Total Human Immunodeficiency Virus Type 1 (HIV-1) DNA Measured in CD4+ T_N, T_{CM}, and T_{TM}+T_{EM} Cells Isolated from HIV-1–infected Individuals on Antiretroviral Therapy

Virological parameter	T_{CM} vs T_N	T_{TM}+T_{EM} vs T_N	T_{CM} vs T_{TM}+T_{EM}
Fold change in IUPM	11.90	12.29	1.03
Fold change in HIV-1 DNA	5.60	13.70	1.75
Difference in IUPM when corrected for difference in HIV-1 DNA	2.13	0.90	0.59

Abbreviations: IUPM, infectious units per million cells; T_{CM}, T central memory cell; T_{EM}, T effector memory cell; T_N, T naive cell; T_{TM}, T transitional memory cell.

Depletion of T_{SCM} Cells From T_N Population Has No Impact on the HIV-1 DNA or Virus Recovery

CD4+ T_{SCM} are thought to be a reservoir of latent HIV-1 in vivo [8]. T_{SCM} and T_N cells express many of the same characteristic surface markers, including CD45RA, CCR7, CD62L, CD28, CD27, IL-7Rα (CD127), and CD95. Therefore, based on our purification protocol, our T_N population would include T_{SCM} cells. We therefore removed T_{SCM} cells from the T_N population by depletion of CD95, which is expressed on T_{SCM} but not T_N cells, in 3 donors (Table 3). Consistent with previous reports, we found that the T_{SCM} population accounted for roughly 5% of the T_N cell population (range, 3.26%–6.28%) [8, 10, 32–35]. We observed no significant differences in total HIV-1 DNA (Table 3), total virus recovery (Table 3), or replication-competent HIV-1 (Figure 4B) in the total T_N population vs the T_N population that was lacking the T_{SCM} cells (T_N [CD95-]).

DISCUSSION

The latent HIV-1 reservoir is frequently described as residing within resting memory CD4+ T cells. This is largely due to the consistent finding that memory CD4+ T cells harbor the highest levels of HIV-1 DNA in individuals on ART [10–13]. This has yielded little research into the contribution of CD4+ T_N cells to the latent reservoir. In 2013 Sáez-Cirión et al reported that in some infected individuals who received ART within 10 weeks of primary infection in the French Virological and Immunological Studies in Controllers After Treatment Interruption cohort, viremia could be controlled for at least 24 months post-treatment interruption [6]. In this patient population, HIV-1 DNA was only detected in CD4+ T_N cells in 2 of 11 individuals, whereas the resting memory CD4+ T-cell subsets (T_{CM}, T_{TM}, and T_{EM}) harbored comparable levels of HIV-1 DNA [6]. This finding suggested to us that the latent HIV-1 reservoir in CD4+ T_N cells may be more important than previously considered and could be a factor that contributes to viral control in these individuals.

Using a primary cell model of HIV-1 latency [14], we previously reported that although T_N cells harbored significantly lower levels of HIV-1 DNA, they produced as many virions as did the T_{CM} cells following latency reversal. Consistent with this in vitro data [14], in this study we show that although the frequency of HIV-1 DNA is lower in T_N compared to T_{CM} cells purified from HIV-1–infected individuals on ART, as much, if not more, virus is produced from the T_N cells following exposure to LRAs (Figure 2). Importantly, we recovered replication-competent HIV-1 from the CD4+ T_N, T_{CM}, and T_{TM}+T_{EM} subsets. While on average higher IUPM values were determined in the T_{CM} and T_{TM}+T_{EM} cells compared to the T_N cells (Figure 4A), once we normalized these values to the number of infected cells, we found minimal differences in IUPM between the different subsets (Table 2). This finding suggests that a higher proportion of the HIV-1 DNA in the T_N cells, compared to the T_{CM} and T_{TM}+T_{EM} cells, may be intact and replication competent. Recently, Hiener et al quantified the frequency of intact proviruses in different T-cell subsets from HIV-1–infected individuals on ART [36] and found a higher proportion of intact proviruses in T_N cells compared to T_{CM} cells. Interestingly, in that study, they found that the highest frequency of intact provirus was in the T_{EM} compartment and that no intact provirus could be recovered from the T_{CM} compartment. However, others have found varying levels of replication-competent virus across CD4+ T-cell subsets, generally finding that T_{CM} cells contain the highest IUPM on a population level; however, this is not consistent across all donors [12, 37]. These differences could be reflective of differences in time of infection prior to ART initiation, time on ART, or other immunological disparities. Further investigation is warranted to better understand differences in T-cell reservoirs between individuals.

In conclusion, our data highlight that quantifying HIV-1 DNA alone may not be predictive of the size of the inducible latent reservoir and further reinforce that CD4+ T_N cells, which are abundant and have exceptionally long half-lives (1–8 years) [38, 39], are an important cellular reservoir of latent HIV-1 infection. There are, however, limitations to our work. First, our sample size was relatively small (n = 7). Second, our work only provided insight into the latent viral reservoir in peripheral blood and not tissue. In regard to the latter, Mavigner et al [40] recently reported that CD4+ T_N cells substantially contributed (approximately 70%–80%) to the

Table 3. Quantification of Human Immunodeficiency Virus Type 1 (HIV-1) DNA and Inducible Extracellular HIV-1 RNA in T_N and T_N(CD95-) Cells Purified From HIV-1–Infected Individuals on Antiretroviral Therapy

	HIV-1 DNA (copies/10^6 cells)		HIV-1 RNA in the Supernatant (copies/mL)											
			Anti-CD3/CD28		Phytohaemagglutinin + Interleukin 2		Phorbol-myristate-acetate + ionomycin		Prostratin		Panobinostat		Romidepsin	
Donor No.	T_N	T_N CD95-	T_N	T_N CD95-	T_N	T_N CD95-	T_N	T_N CD95-	T_N	T_N CD95-	T_N	T_N CD95-	T_N	T_N CD95-
5	1232	1249	12 670	5460	16 100	5930	2220	1042	2465	1615	0	0	0	0
6	158	173	0	0	0	0	0	0	0	0	0	0	0	0
7	1380	1112	13 700	12 100	11 700	9050	17 400	15 250	15 860	12 280	298	61	54	144
Mean	923	845	8790	5853	9267	4993	6540	5431	6108	4632	99	20	18	48

Abbreviations: T_N, T naive cell.

total CD4+ reservoir of Simian immunodeficiency virus (SIV) infection in both blood and lymph nodes of ART-suppressed rhesus macaques infants. In contrast, the SIV reservoir in CD4+ T_N cells in the peripheral blood of ART-suppressed adult rhesus macaques was much lower (14%) but still contributed significantly to the reservoir in lymph nodes (approximately 40%–60%).

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. J. M. Z., J. W. M., and N. S. C designed the study. J. M. Z., D. M., and M. S collected the data. J. M. Z. Manuscript did the statistical analysis. J. M. Z. and N. S. C. wrote the manuscript. All authors reviewed and approved the final manuscript.

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Potential conflicts of interest. J. W. M. is a consultant to Gilead Sciences and a shareholder of Co-Crystal, Inc. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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