A subgroup of Ugandan elite and viremic controllers naturally control HIV-1 infection by blocking R5-tropic viral entry.

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Abstract

Background: World over, there are antiretroviral therapy naïve individuals infected with HIV who maintain their CD4+T cell count above 500 cells/µl over 7-10 years and viral loads well controlled below undetectable levels (termed elite controllers, ECs) or at least 2,000 copies/mL (termed viremic controllers, VCs) for at least 12 months. Mechanisms responsible for HIV control in these individuals have not been fully elucidated. We hypothesized that CD4+T cells from elite and viremic controllers are naturally resistant to HIV-1 infection by blocking R5-tropic viral entry. We conducted a case-controlled study in which archived peripheral blood from 31 ECs/VCs and 15 progressors were investigated using *in vitro* HIV-1 infectivity assays.

Results: Briefly, we purified CD4+T cells from peripheral blood using EasySep CD4+ positive selection kit followed by CD4+T cell activation using IL-2, anti-CD28 and anti-CD3. Three days post-activation, CD4+T cells were spinoculated and co-cultured with vesicular stomatitis virus G (VSV-G)-pseudotyped HIV, R5 (ADA-enveloped)- and X4 (NL4.3-enveloped v)-tropic HIV-1. Three days post infection, we quantified and compared the percentage infection of CD4+T cells in cases and controls. We demonstrate that a subgroup of Ugandan elite and viremic controllers possess CD4+T cells that are specifically resistant to R5-tropic virus, remaining fully susceptible to X4-tropic virus.

Conclusion: Our study suggests that a subgroup of Ugandan elite and viremic controllers naturally control HIV-1 infection by blocking R5-tropic viral entry. Further research is needed to explore mechanisms of HIV control in the African population.

Background

Before widespread use of highly active antiretroviral therapy (HAART), the median duration from HIV seroconversion to full brown AIDs was approximately 8–10 years (1, 2). Without HAART, the natural history of HIV-infection was marked by the development of AIDS-defining illness in more than 99% of cases(3). It was also noted that a few individuals showed little or no clinical progression, later termed as Long-Term Non-progressors (LTNPs) (2). In the western world, these individuals comprise about 3–5% of the HIV-infected population(4). There is paucity of literature about adult LTNPs in sub-Saharan Africa and the prevalence is still unknown(5). Recent data from Rakai HIV cohort in Uganda reported a prevalence of 9.1% LTNPs(6).

Two phenotypes of LTNPs have been recently described. The first phenotype includes LTNPs who naturally control HIV-1 replication, with undetectable plasma viral loads termed elite controllers (EC). They contribute less than 1% in western HIV cohorts and 1.4% in Ugandan HIV cohorts (7). The second phenotype includes LTNPs who maintain their plasma viral loads between 20 and 2,000 copies/mL, termed viremic controllers (VC) (8). In our recent study, conducted in a large urban HIV clinic in Kampala, Uganda, we reported a prevalence of 0.26% (38/14,492) of HIV controllers. We identified 36 ECs and 2 VCs. These individuals were middle-aged with an average CD4 count of 858 (95% CI: 795–921)(6).

LTNPs are aggressively being studied to determine the mechanisms responsible for spontaneous virologic control with the hope of developing novel treatment strategies and perhaps a therapeutic vaccine (4). Mechanisms leading to spontaneous virologic control have not been fully described. Those described account for only 20% of LTNPs (2). Current studies using western HIV cohorts have demonstrated CD4 + T cell intrinsic resistance to R5-tropic HIV strains in contrast to X4-tropic HIV(9, 10). It appears most likely that long term non-progressor status depends highly on the tropism of the virus. However, other studies have yielded conflicting results (11, 12). In the African HIV-infected population, it still unknown whether LTNPs exhibit intrinsic resistance to R5-tropic entry as demonstrated in the western cohorts(2). We therefore sought to determine the susceptibility of Ugandan LTNP-derived-CD4 + T cells to HIV-1 infection using *in vitro* HIV-1 infectivity assays

Results

CD4 + T cell purification, activation and infection with VSVG enveloped HIV-1

To perform single-cycle HIV-1 infection assay on CD4 + T cells of cases and controls, we first purified CD4 + T cells from PBMCs of 31 cases and 15 controls using EasySep CD4 + T cell enrichment kit following manufacturer's instructions. The percentage purity of CD4 + T cells was comparable in both cases and controls at ≥95% (Fig. 1A). Next, we activated purified CD4 + T cells using IL-2, anti-CD3 and anti-CD28. Three days post-activation, we compared the percentage expression of CD25 and CD69 on activated CD4 + T cells as well as the percentage of CD4 + T cells with forward scatter (FSC) above 200 among cases and controls. CD4 + T cells from cases were highly activated compared to controls (Fig. 1B, 1C, 1D).

Several investigators have demonstrated that HIV-infection of CD4 + T cells is increased upon T cell activation(13). To determine if optimal CD4 + T cell activation was achieved to ensure successful viral infectivity assays, we performed single-cycle HIV-1 infection assay on CD4 + T cells of cases and controls using VSVg-enveloped HIV-1. Next, we performed linear regression and spearman's rank correlation analyses between CD4 + T cell activation and percentage infection by VSVg-enveloped virus. We observed a positive correlation ($r = 0.5877$, $p < 0.0001$) (Fig. 1E, 1F, 1G).

Based on this finding, we set a threshold value of 5% CD4 + T cell activation (based on FSC > 200) and 5% VSVg-enveloped viral infection for cases and controls to be included in our final analytical sample for other viral infectivity assays performed. Among cases, a final analytical sample size of 17 was considered excluding 13 samples (10 had less than 5% T cell activation and 3 had less than 5% T cell infection with VSVg-enveloped virus). Similarly, a final analytical sample size of 4 was considered among controls (9 had less than 5% T cell activation and 3 had less than 5% T cell infection with VSVg-enveloped virus). (See Fig. 2)

Susceptibility of CD4 + T cell to R5 and X4-tropic single cycle HIV-1 strain

In order to examine CD4 + T cell susceptibility to HIV-1 infection in cases and controls, we performed further single-cycle HIV infection assays using R5-tropic virus(ADA-enveloped HIV-1) and X4-tropic virus (NL4.3-enveloped HIV-1). CD4 + T cells from cases and controls were fully susceptible to VSVg- and NL4.3-pseudotyped viruses with no statistically significant differences (Fig. 3A and 3B).

For ADA-pseudotyped virus, we observed a mixed picture. CD4 + T cells among four cases (EC-3,8,30,36) exhibited reduced entry of ADA-pseudotyped virus compared to controls (Fig. 3C and 4A), while other ECs showed uptake of ADA-enveloped virus.

In a post-hoc analysis, it was revealed that CD4 + T cells from a subset of four cases exhibited relative resistance to ADA-pseudotyped virus, in comparison to the remaining ECs and controls. In contrast, these T cells were fully susceptible to NL4.3-enveloped HIV (Fig. 3D). Because of limited sample availability, we repeated infectivity assays only for EC008. Results were consistent (Figure .4B)

Discussion

The quest for mechanisms responsible for virologic control in long term non progressors (LTNPs) has intensified in the western HIV cohorts. However, these mechanisms have not been fully described especially in the African population. In this study, we hypothesized that CD4 + T cells derived from adult LTNPs are naturally resistant to HIV-1 infection by blocking R5 tropic HIV-1 entry.

Our results suggest that a subset of LTNPs possess CD4 + T cells that are specifically resistant to R5-tropic HIV-1 infection. We demonstrated that CD4 + T cells purified from 4 out of 31 LTNP samples were resistant to R5-tropic HIV but remained susceptible to X4-tropic and VSV-G pseudotyped HIV. The remaining cases in our study most likely accomplish HIV control via other mechanisms that may be CD4 + T cell extrinsic.

In this study, we demonstrated that approximately 13% of LTNPs in a Ugandan HIV cohort harbor CD4 + T cells which are resistant to R5 tropic HIV-1 entry. This is low compared to the Western HIV cohorts where investigators have reported up to 25% of LTNPs harboring intrinsically resistant CD4 + T cells(9). A variety of reasons might attempt to account for this difference: (1) it is likely that a significant proportion of LTNPs with a peripheral CD4 + T cell count < 500/μl had already been initiated on HAART and thus excluded in this study. (2) Because of the newly launched test and treat policy in Uganda, a majority of potential LTNPs with CD4 + T cell > 500/μl had already been started on HAART(6). (3) In addition, because many HIV controllers in the developed world have CD4 + T cell count < 500/μl, setting a threshold CD4 count of > 500/μl would have excluded perhaps even a greater number of Ugandan HIV controllers.

In vitro R5 tropic HIV-1 resistant phenotype has been reported in some but not other studies. In one study supporting our findings, Chen demonstrated low levels of p24 antigen production after activation of LTNP- derived CD4 + T cells with IL-2/anti-CD3 followed by exogenous HIV-1 infection with the primary R5-utilizing strain 91US056(14). Similarly, in another study, Sáez-Cirión demonstrated low HIV-1 susceptibility of anti-CD3-activated CD4 + T cells from LTNPs further supporting our findings(15). In their

recent publication, Gonzalo-Gil and colleagues demonstrated R5-tropic resistance in 25% of their study population. Common to all these studies is the utilization of similar activation protocols with anti-CD3 antibodies which stimulate CD4 + T cells via T cell receptor (TCR) signaling. This closely reflects the conditions in in vivo HIV-1 infection(14). Other studies have used alternative activation protocols for instance phytohemagglutinin (PHA)

However, other studies report contradictory findings. In one study, Rabi demonstrated that purified CD4 + T cells from fresh peripheral blood of LTNPs were susceptible to HIV-1 entry and productive infection(16). Assays performed on 17 LTNPs could not rule out a possibility of detecting a resistant phenotype in the population studied. It is possible that all their LTNPs were susceptible to HIV-1. Another point to note is that, the investigators did not assay for markers of activation making it hard to ascertain whether CD4 + T cells were activated or not(16). It is possible that purified CD4 + T cells from LTNPs in this study were activated *in vivo* increasing susceptibility to HIV-1. Wang also reported that highly purified CD4 + T cells from a single LTNP, activated by PHA were susceptible to HIV-1 infection(11). Because PHA induces a robust polyclonal activation of CD4 + T cells way above physiological levels, it is highly unlikely that the hyper activation observed in vitro appropriately reflects the condition in in vivo HIV-1 infection(16). Other factors have been reported to account for controversial findings. These include: (1) varying physiological state of the cells, (2) viral strains used, (3) viral titers, (4) cell density, (5) cell purity, (6) frequency of medium change, and (7) spinoculation in the experiments(10). In our study, cell purity was judged to be above 96% in both cases and controls by flow cytometry. We also ensured that cells were optimally activated by setting an activation threshold of > 5% for both cases and controls. We also used optimal viral concentration of 1 ml according to Walker and colleagues(10).

Interpretation of our findings must put the following into consideration. Our sample size and volume of collected samples were limited due to launching of the test and treat policy throughout the country at all HIV clinics in 2016. Due to limited sample volumes, we were able to perform single experiments for each of the samples from cases and controls tested. We used pseudotyped viruses that are only capable of a single cycle of infection. While this leads to quantitative measurement of viral infection, it does not measure the events in the viral life cycle that occur post entry other than GFP expression. It is thus possible that there are differences in HIV-1 budding from CD4 + T cells from LTNPs versus progressors. In addition, we did not explore other reported factors like CCR5 expression profile, HLA type, and cytotoxic T/ Natural Killer cellular responses in our study. Our study was also limited by a finite sample size due to changes in HIV treatment guidelines endorsed by the World Health Organization in 2016.

Conclusion

Our study suggests that a subgroup of Ugandan elite and viremic controllers naturally control HIV-1 infection by blocking R5-tropic viral entry.

Recommendations: Further research is needed to explore mechanisms of CD4+T cell resistance to R5-tropic HIV in the African population. In addition, our findings support continued investigation of

coreceptors as a target for drug development and functional eradication of HIV.

Materials And Methods

Study design and Population

A case controlled study was conducted in which archived blood samples from the “Investigation of host genetic factors for HIV control in Ugandan Elite and Viremic controllers (ELITE) study” were used(6). Among its target population, the ELITE study recruited 42 HIV-infected participants’ ≥18 years(6). The participants were ART naïve by 31st December 2015 and had been under care at Makerere University Joint AIDS Program (MJAP), Mulago ISS clinic for at least 5 years with documented serial CD4+T cell counts ≥ 500cells/ml and viral load < 2,000 copies/ml. Their average duration in care at the clinic was 7.36 years (95% CI 6.58-8.13)(6). Of these participants, 36 were ECs, 2 were VCs and 4 were progressors. In addition, ELITE study recruited 15 HIV-infected individuals ≥18 years with documented CD4+T count below 500 cells. Peripheral blood mononuclear cells (PBMCs) were harvested and frozen in liquid nitrogen in the Immunology laboratory of Makerere University College of Health Sciences located on the 1st floor of the Clinical Research building. Figure 2 summarizes the flow of *in vitro* assays performed on PBMCs from 31 LTNPs and 15 progressors.

Ethical considerations

We conducted our study in compliance with recognized international standards, including the International Conference on Harmonization (ICH) and the guidelines of the Declaration of Helsinki. We obtained written informed consent from all study participants. Ethical approval was granted by the Makerere University School of Biomedical Sciences, Higher Degrees Research and Ethics Committee (SBS-HDREC) and the Uganda National Council for Science and Technology in Kampala, Uganda.

Laboratory Methods

PBMC thawing: Aliquots of PBMCs from LTNPs and progressors were removed from liquid nitrogen followed by thawing by a brief incubation of the vials in a 37^{o}C water bath until there was a small ice pellet left. Cells were washed immediately with thawing medium composed of 90% RPMI 1640 medium (Thermofischer Scientific, Grand Island USA, catalogue no. 11875093) and 10% Fetal Bovine Serum (Thermofischer Scientific, South America, catalogue no. 10270106) in a 15ml centrifuge tube.

Enrichment/purification of CD4+ T cells: CD4+T cell enrichment was performed using EasySep™ Human CD4+T Cell Isolation Kit (Stem Cell Technologies, Catalog no. 17952). Briefly, a cell suspension was prepared at a concentration of 4 x 10^8 cells/mL in 1.6ml PBS/10% FBS using 5ml capacity round bottomed polystyrene falcon tubes. EasySep™ Dextran Rapid Spheres were added to the sample at a concentration of 50 µl /mL. PBS/10%FBS was added to top up the sample up to 2.5mL. The mixture was then mixed by gently pipetting up and down 2-3 times and placed the tube (without lid) into the EasySep magnet and incubated for 3 minutes at room temperature. The magnet was then picked up, and in one

continuous motion inverted with the tube, pouring the enriched cell suspension into a new falcon tube. To assess CD4+T cell purity, samples were stained with 5µg of mice anti-human CD4-allophycocyanin (APC) (RPAT4 clone), (BioLegend, San Diego, CA, USA, catalogue no. 300552) and flow cytometry was then performed with a FACS Calibur H (Becton Dickinson, Franklin Lakes, NJ) (Figure 5A illustrates the gating strategy used).

HIV-1 pseudo viruses used for the infectivity assays: Three strains of HIV-1 pseudovirus were used in this experiment i.e (1) **strain I**: vesicular stomatitis viral glycoprotein (VSV-G) enveloped HIV-1, an HIV-1 pseudotyped virus encoded with a gene reporter-green fluorescent protein (GFP). We used VSV-G enveloped HIV-1 as a positive control to demonstrate that purified and activated CD4+T cells from cases and controls were in good condition to take up HIV-1. (2) **strain II:** ADA enveloped HIV-1 pseudotyped virus particles, a wild type R5-tropic HIV-1 subtype B isolate which utilizes CCR5 as a coreceptor and **strain III**: NL4.3, an X4-tropic HIV. The three HIV strains were obtained from Dr Richard Sutton laboratory at Yale University, USA.(9)

Titration of HIV-1 pseudovirus: The infectious dose of HIV-1 particles had been standardized by titration protocols in the Sutton Laboratory at Yale University(9, 10). We therefore adopted and used the optimal dose of 1ml of the virus as previously described in GHOST Hi5 and CD4+T cells in the Sutton Laboratory. A dose of 1ml gave a maximum infectivity without compromising the viability of the CD4+T cells(9, 10).

***In vitro* CD4+T Cell activation:** EasySep enriched CD4+T cells (200,000 cells per well) were activated in tissue culture as follows. 10µg of anti-CD3 (OKT3 clone, eBioscience™, San Diego, CA, Catalog no.14-0037-82) in 1 ml of cold PBS was coated on a 96 well plate. After overnight incubation at 4^{o}C, the plate was washed three times with cold PBS. A solution of 200IU/ml of IL-2 and 10µg/ml of mouse anti-human-CD28 (Clone L293, BD biosciences, catalogue no. 348040) in 1 ml of RPMI/10%FBS was added to the coated plate. 200,000 cells were added to the well and incubated for 3 days.

The activation state of CD4+T cell was monitored daily by microscopy for phenotypic signs (clumping and increase in size). Three days post activation, CD4+T cell size was measured based on Forward scatter (FSC) using flow cytometry. The percentage CD4+T cells with FSC>200 were computed. In addition, we measured the expression of CD69 and CD25 on CD4+T cells three days post-infection using flow cytometry. Briefly, samples were stained with 5µg/ml of mouse anti-human CD69- fluorescein isothiocyanate (FITC) (BD biosciences, catalogue no 555530) and 5µg of anti-human CD25- pyridine chlorophyll protein (PerCP) (BD bioscience catalogue no. 560503). Flow cytometry analysis was then performed with a FACS Calibur H (Becton Dickinson, Franklin Lakes, NJ). Figure 5B illustrates the gating strategy used. To confirm CD4+T cell activation, we based on the level of expression of CD25 and CD69 on CD4+T cells from cases and controls. The choice of CD25 and CD69 as our markers of T cell activation was based on prior experiments of HIV-1 infectivity assays reported by Walker and colleagues(10)

Infection with HIV pseudoviruses: Activated CD4+T cells were then cultured in M24 plates separately with 1ml of each of the three strains of pseudotyped HIV particles for 3 days. A third culture was setup in which no virus was added to the activated cells (Negative control). After 3 days of CD4+T cell-pseudotyped virus co-culture, we measured percentage GFP/YFP positive cells by flow cytometry using FACS Calibur H (Becton Dickinson, Franklin Lakes, NJ). In brief, we collected CD4+ T cell from the culture plate by pipetting up/down. We transferred cells into flow tubes with 50µl of fixation buffer (2% paraformaldehyde, PFA in PBS) and analyzed percentage GFP/YFP positive cells with flow cytometer. Figure 6 illustrates the gating strategy used.

Biostatistical analysis

Data was analyzed using flow Jo version 9.3.2 (Tree Star Inc., Ashland, OR) and GraphPad prism version 8.0**.** Data was graphically presented using column scatter plots for cases and control groups displaying group means and standard deviations or medians and ranges. We compared means/medians of the cases and control group. Statistical comparison was done using Mann Whitney U test for two groups and Wilcoxon rank sum test for more than two groups. Statistical significance was considered if p-value $\leq$ 0.05.

List Of Abbreviations

ADA: Adenosine deaminase glycoprotein

CCR5: Chemokine receptor 5

CXCR4: Chemokine receptor 4

EC: Elite controller

GFP: Green Fluorescent protein

HLA: Human Leukocyte antigen

M-CSF Macrophage colony-stimulating factor

MHC: Major histocompatibility complex

PBS Phosphate buffered saline

RPMI Roswell Park Memorial Institute

VC: Viremic controller

VSV-G: Vesicular stomatitis virus glycoprotein

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the Makerere University School of Biomedical Sciences, Higher Degrees Research and Ethics Committee (IRB NO: SBS-HDREC-372) and the Uganda National Council for Science and Technology in Kampala, Uganda. All participants consented to participate in the study and allowed to use their blood sample in this investigation.

Consent to publish

All authors agreed to publish this work.

Availability of data and materials

A dataset for CD4+T purification, activation and infectivity assays has been attached in table.1

Competing interests

All authors declare no competing interests.

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Authors' contribution

Alex Kayongo: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Data curation, Formal analysis, Original draft writing and editing; Anxious Niwaha Jackson, Derrick Semugenze and Mary Nantongo : Investigation and Methodology; Robert Kalyesubula: Investigation, Methodology and Project administration; Bernard S Bagaya: Investigation, Methodology and Project administration; Moses L Joloba: Conceptualization, Mentorship, Investigation, Design, Funding acquisition and drafting the manuscript for important intellectual content

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Table

Table 1: Dataset for CD4+T purification, activation and infectivity assays

Sample ID	CD4 purity(%)	%CD4+T (FCS>2	CD25(%)	CD69(%)	% GFP - control	% GFP (VSVg)	% GFP(ADA)	% GFP (NL4-3)
C001	99.2	20.30	46.80	24.50	0.00	0.00	0.00	0.00
C013	99.3	17.40	0.73	0.18	0.00	22.10	0.49	1.20
C003	98.6	0.03	20.10	9.96	0.00	0.00	0.00	5.56
C004	98.4	0.12	2.18	2.18	0.00	20.00	0.00	0.00
C012	98.5	0.03	0.00	0.00	0.00	0.00	0.00	0.00
C002	98.6	0.03	11.40	1.04	0.00	0.00	0.00	0.00
C005	98.5	1.62	33.90	17.90	0.00	16.70	0.00	0.00
C006	98.0	0.13	13.50	7.69	0.00	0.00	0.00	0.00
C007	99.4	8.77	41.30	18.20	0.00	0.00	0.00	0.00
C008	98.8	2.43	6.45	9.68	0.00	0.00	0.00	0.00
C009	99.1	0.05	4.00	2.29	0.00	0.00	0.00	0.00
C010	99.5	0.06	4.02	1.60	0.00	0.03	0.03	0.00
C014	98.0	5.32	64.30	49.30	0.00	7.19	0.47	0.28
C011	98.2	56.60	45.40	48.10	0.00	8.87	0.35	0.08
C012	98.5	61.50	63.40	26.50	0.00	45.30	0.24	0.19
EC001	99.4	61.80	34.20	20.40	0.01	34.70	0.25	0.57
EC002	99.1	58.30	36.80	7.89	0.02	9.97	0.54	0.66
EC003	99.2	26.30	41.30	40.70	0.00	30.70	0.03	0.15
EC004	99.4	17.50	79.10	26.20	0.22	5.77	0.49	0.38
EC007	99.4	74.60	71.40	33.50	0.00	45.50	0.26	0.25
EC008	97.6	54.70	16.80	38.40	0.00	15.30	0.10	0.40
EC009	98.3	31.20	65.10	36.20	0.00	7.15	0.41	0.36
EC011	98.3	48.50	65.90	55.20	0.00	5.34	0.18	0.09
EC012	98.3	53.80	70.40	41.60	0.00	9.84	0.25	0.24
EC013	98.9	40.40	23.00	47.70	0.00	18.00	0.17	0.44
EC014	96.5	25.80	58.50	45.00	0.00	9.44	0.48	0.50
EC017	99.8	1.44	39.90	25.80	0.00	1.54	0.00	0.00
EC018	99.5	0.97	1.53	0.76	0.58	0.50	1.27	7.56
EC020	99.4	5.15	22.20	27.30	0.00	4.32	0.14	0.00
EC021	99.2	10.20	13.20	15.70	0.09	27.20	0.06	0.39
EC022	98.7	0.55	6.03	10.80	0.00	2.80	4.00	9.64
EC023	98.3	23.00	10.30	9.21	0.00	12.10	3.96	1.86
EC024	99.5	0.11	0.99	0.59	0.00	0.00	0.00	0.00
EC025	96.8	0.79	4.27	3.05	0.00	3.51	1.75	0.00
EC030	99.7	12.20	28.90	24.50	0.00	18.00	0.00	0.22
EC031	100.0	4.12	23.80	15.80	0.00	0.00	0.00	0.00
EC032	98.5	0.29	7.37	5.26	0.00	13.80	0.00	5.71
EC033	99.9	2.00	27.30	11.40	0.00	4.04	0.00	0.00
EC034	98.6	2.22	13.70	9.96	0.00	5.71	0.00	0.00
EC035	99.0	0.99	3.20	1.60	0.00	0.00	0.00	0.00
EC036	98.8	27.40	51.50	49.60	0.25	10.10	0.00	0.74
EC037	98.9	1.34	36.70	37.40	0.00	5.00	0.00	0.00
EC038	96.9	51.80	46.50	47.20	0.00	16.40	0.29	0.34
EC040	99.2	18.80	31.40	33.90	0.00	33.80	0.30	0.19
EC041	99.3	25.00	51.50	39.40	0.00	0.00	0.00	0.00
EC042	99.1	25.00	49.50	30.80	0.00	0.00	0.00	0.00

A dataset for CD4+T purification, activation and infectivity assays. C-controls, FSC-Forward scatter, GFP=Green Fluorescent Protein, VSVG: VSVg enveloped virus, ADA: ADA-enveloped virus, NL4.3: NL4.3-enveloped virus.

Figures

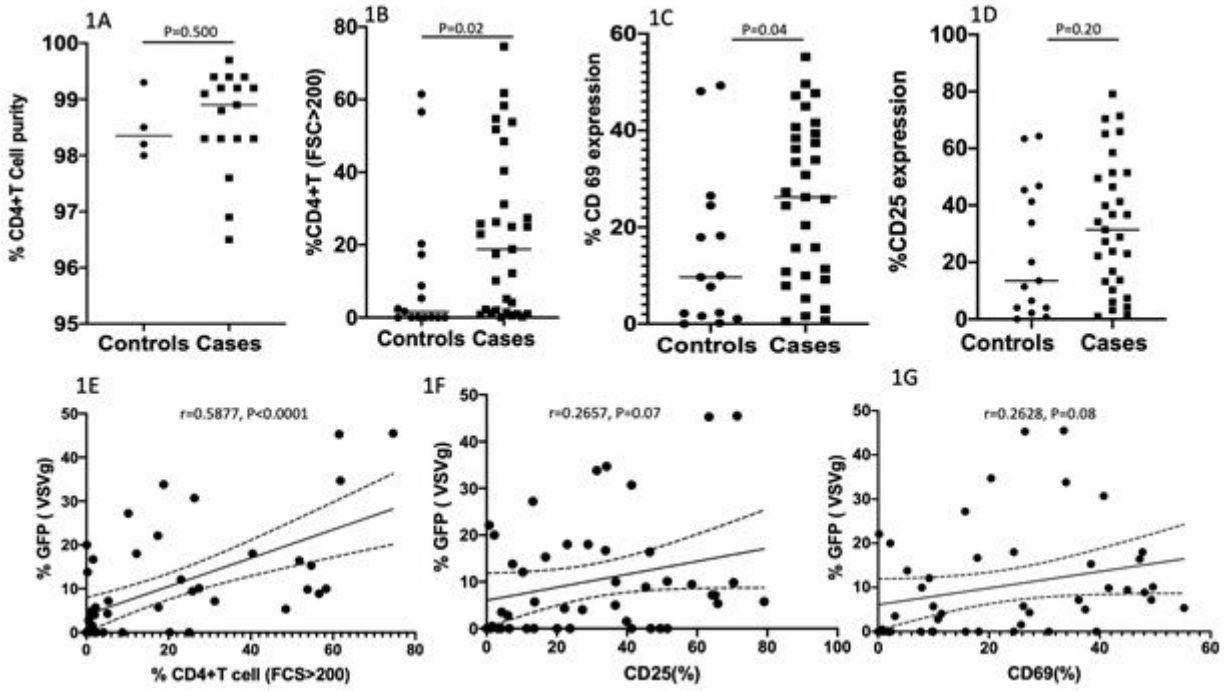

Figure 1

Results from CD4+T cell purification, activation and infection with VSVg-enveloped HIV-1. 1A: CD4+T cell enrichment was performed using EasySep™ Human CD4+T Cell Isolation Kit. To assess CD4+T cell purity, samples were stained with 5µg of mice anti-human CD4-allophycocyanin (APC) and flow cytometry was then performed with a FACS Calibur H. CD4+T cell purity was compared between cases and controls using Mann Whitney U test. Number of cases=31, controls=15, alpha=0.05. 1B: EasySep enriched CD4+T cells were activated in tissue culture using IL-2, anti-CD3 and anti-CD28. Three days post activation, CD4+T cell size was measured based on Forward scatter (FSC) using flow cytometry. The percentage CD4+T cells with FSC>200 were computed and compared between cases and controls using the Mann Whitney U test. Number of cases=31, controls=15, alpha=0.05. 1C-D: Three days post activation, samples were stained with 5µg/ml of mouse anti-human CD69- fluorescein isothiocyanate (FITC) and 5µg of anti-human CD25- pyridine chlorophyll protein (PerCP). Percentage CD69 and CD25 expression were quantified using flow cytometry. CD69/CD25 expression were compared between cases and controls using Mann Whitney U test. Number of cases=31, controls=15, alpha=0.05. 1E-G: Activated CD4+T cells were cultured in M24 plates with 1ml of VSVg-pseudotyped HIV particles for 3 days. Three days post infection, percentage GFP positive cells was quantified by flow cytometry using FACS Calibur H. We performed linear regression and spearman's rank correlation analysis to investigate the relationship

between viral infection and CD4+T cell activation i.e CD4+T cell size, CD69 and CD25 expression. Number of cases=31, controls=15, alpha=0.05.

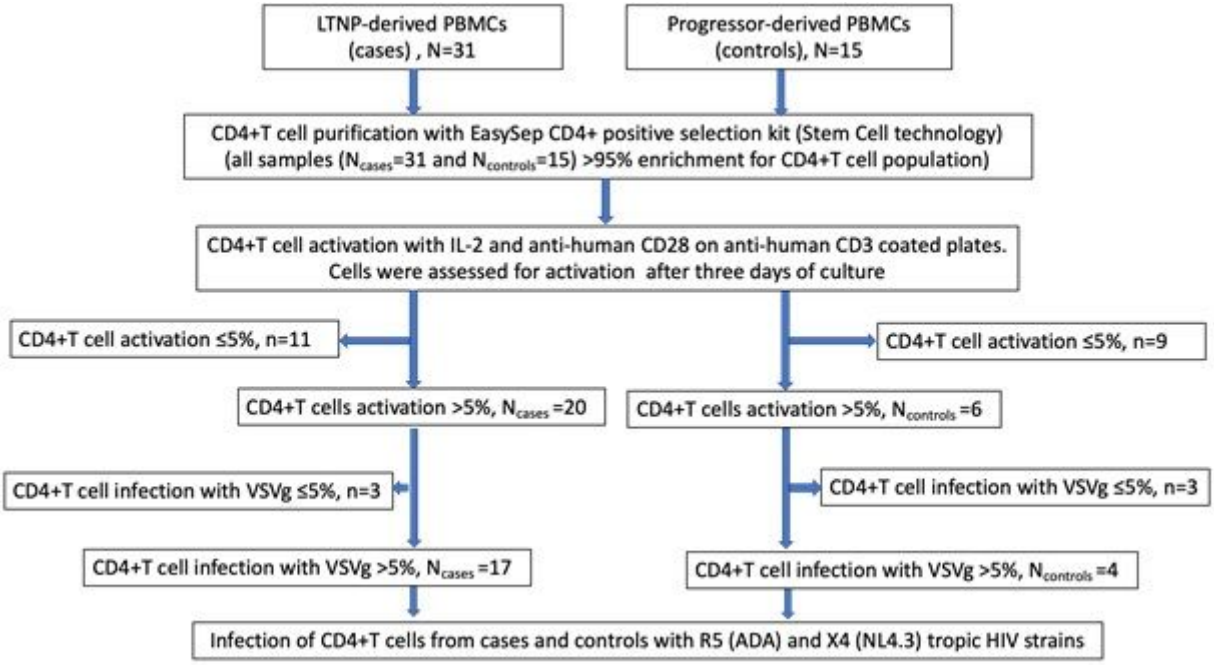

Figure 2

Flow chart summarizing in vitro assays performed on PBMCs obtained from 31 LTNPs (cases) and 15 progressors (controls). Summarizes the flow of in vitro assays performed on PBMCs from 31 LTNPs and 15 progressors. The percentage CD4+T cell purity was comparable in both cases and controls at ≥95%. Following stimulation of CD4+T cells with IL-2 and anti-CD3/28, T cells from cases were highly activated compared to controls. A threshold value of 5% CD4+T cell activation and 5% VSVg-enveloped viral infection was set for both cases and controls to be included in the final analytical sample size for viral infectivity assays with R5 and X4-tropic viruses. Among cases, a final analytical sample size of 17 was considered excluding 13 samples (10 had less than 5% T cell activation and 3 had less than 5% T cell infection with VSVg-enveloped virus). Similarly, a final analytical sample size of 4 was considered among controls (9 had less than 5% T cell activation and 3 had less than 5% T cell infection with VSVg-enveloped virus). This was informed by a positive correlation between CD4+T cell activation and VSVg-envelope viral infectivity.

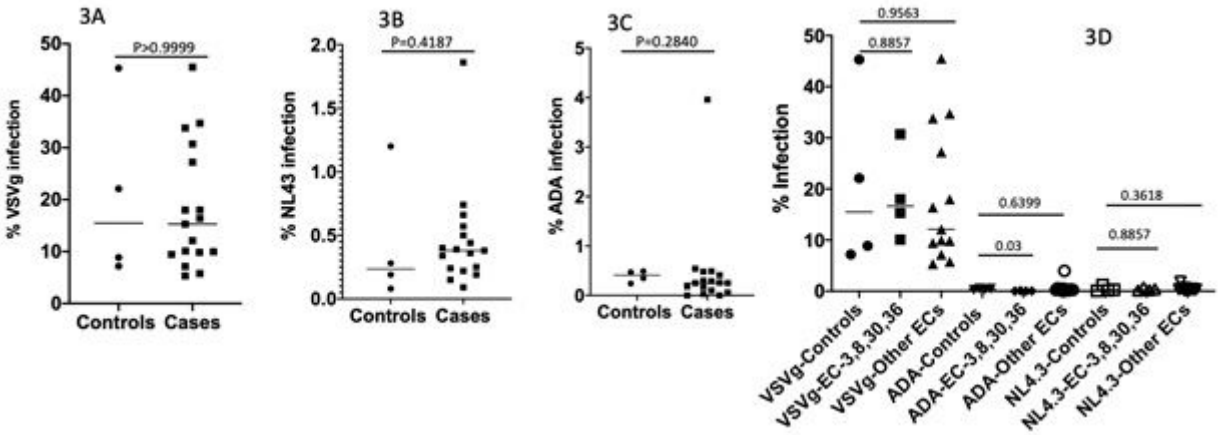

Figure 3

Susceptibility of CD4+T cell to pan-tropic, R5 and X4-tropic single cycle HIV-1 strain. 3A-C: Activated CD4+T cells were cultured in M24 plates separately with 1ml of each of the three strains of pseudotyped HIV particles for 3 days. Three days post-infection, we measured percentage GFP positive cells by flow cytometry using FACS Calibur H. We compared percentage CD4+T cell infection by each of the strains among cases and controls using Mann Whitney U test (5A,5B and 5C). Cases=17, controls=4, alpha=0.05. 3D: In a post-hoc analysis, it was revealed that CD4+T cells from a subset of four cases (EC-3,8,30,36) exhibited relative resistance to ADA-pseudotyped virus, in comparison to the remaining ECs and controls. In contrast, these T cells were fully susceptible to VSVg- and NL4.3-enveloped HIV (Figure 5D).

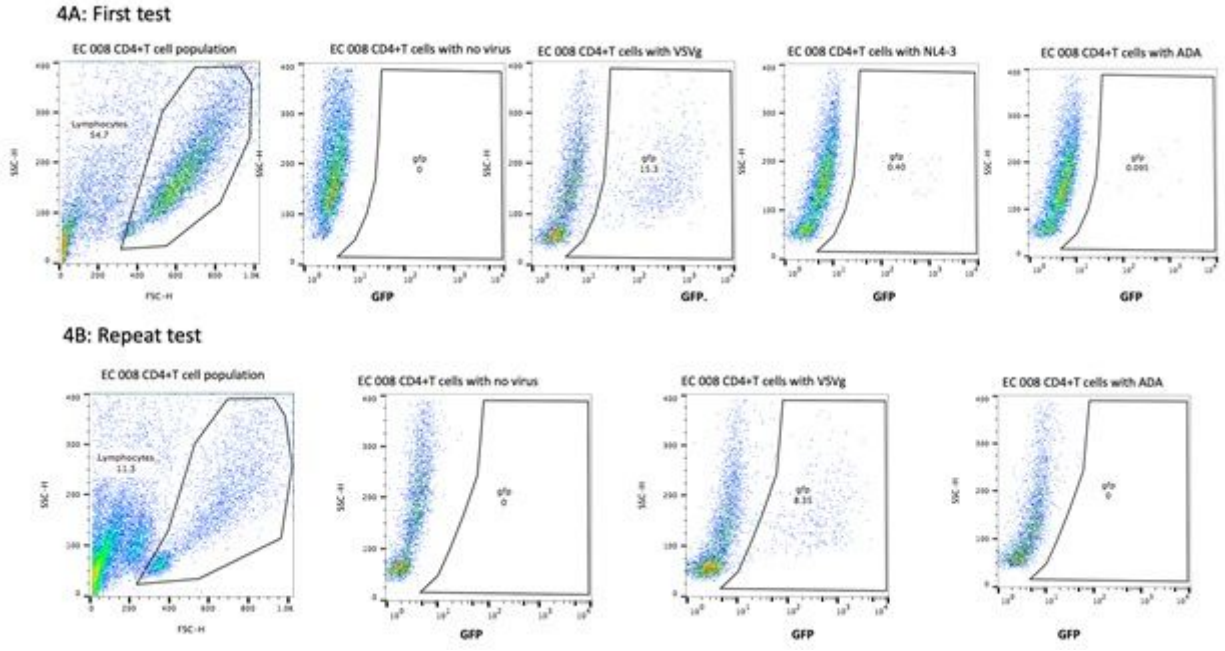

Figure 4

Viral Infectivity assays for EC008. Activated CD4+T cells from EC 008 were cultured in M24 plates separately with 1ml of each of the three strains of pseudotyped HIV particles for 3 days. A third culture was setup in which no virus was added to the activated cells (Negative control). After 3 days of CD4+T cell-pseudotyped virus co-culture, we measured percentage GFP positive cells by flow cytometry using FACS Calibur H.

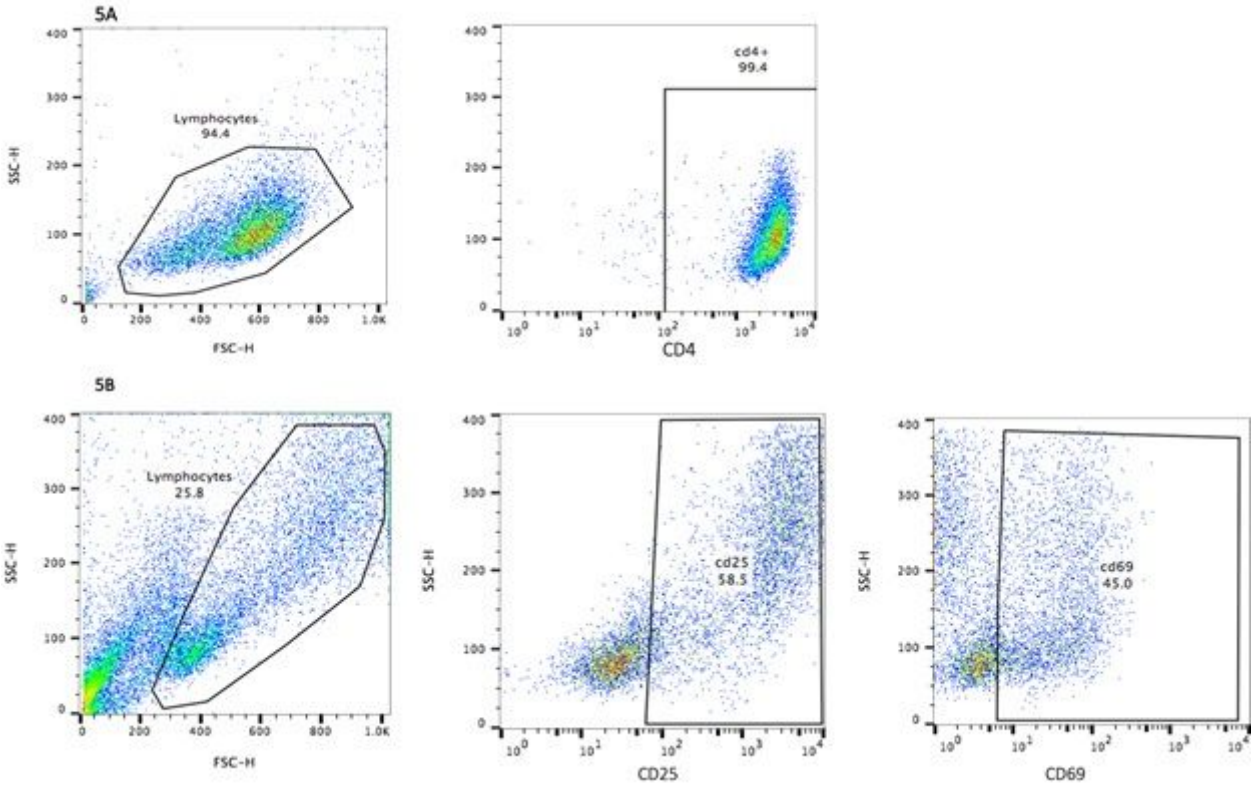

Figure 5

Gating strategy used to quantify percentage purity and activation of CD4+T cells.5A: CD4+T cell enrichment was performed using EasySep™ Human CD4+T Cell Isolation Kit. To assess CD4+T cell purity, samples were stained with 5µg of mice anti-human CD4-allophycocyanin (APC) and flow cytometry was then performed with a FACS Calibur H. 5B: To quantify CD4+T cell activation status, samples were stained with 5µg/ml of mouse anti-human CD69- fluorescein isothiocyanate (FITC) and 5µg of anti-human CD25- pyridine chlorophyll protein (PerCP). Flow cytometry analysis was then performed with a FACS Calibur H.

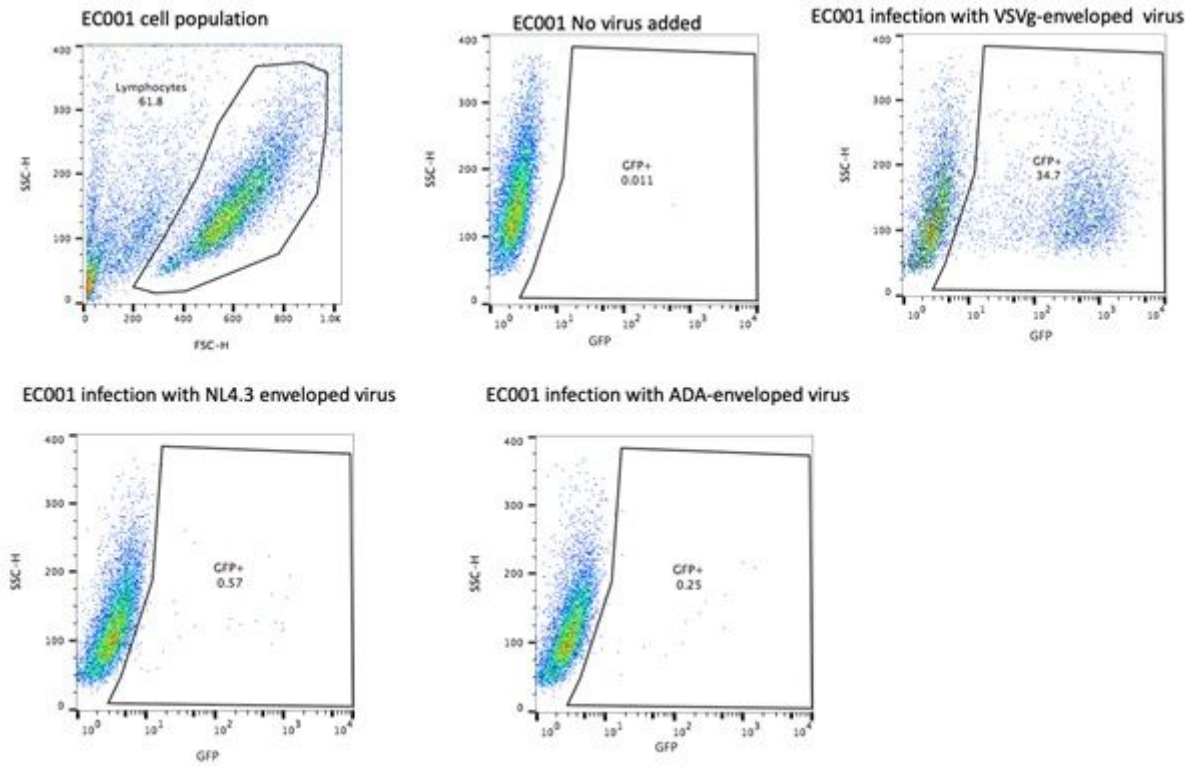

Figure 6

Gating strategy for quantifying CD4+T cell infection with HIV pseudotyped particles. Activated CD4+T cells were cultured in M24 plates separately with 1ml of each of the three strains of pseudotyped HIV particles for 3 days. A third culture was setup in which no virus was added to the activated cells (Negative control). After 3 days of CD4+T cell-pseudotyped virus co-culture, we measured percentage GFP positive cells by flow cytometry using FACS Calibur H.