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Dendritic cell-mediated HIV-1 infection of T-cells demonstrates a direct relationship to plasma viral RNA levels

Reetakshi Arora¹, Lara Bull², Edward B. Siwak¹, Rajesh Thippeshappa¹, Roberto C. Arduino², and Jason T. Kimata^{1,*}

¹ Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030 USA

² Section of Infectious Diseases, Department of Medicine, University of Texas Health Science Center at Houston School of Medicine, Houston, TX 77030 USA

Abstract

Objective—To examine the relationship between infectivity of HIV-1 variants in dendritic cell-mediated *in trans* infection of T-cells and plasma viral RNA levels in infected subjects.

Methods—HIV-1 was isolated from PBMCs of chronically infected individuals, typed for coreceptor usage, and viral replication was examined in monocyte derived-dendritic cells-peripheral blood lymphocytes (DC-PBL) co-cultures. The rate of p24 antigen production during the logarithmic phase of viral replication was determined by ELISA. Additionally, *nef* variants were cloned and expressed *in trans* with a HIV-luciferase vector and CCR5-tropic HIV-1 envelope, and infectivity was measured in DC-mediated capture-transfer assays.

Results—Replication capacity of HIV-1 viral CCR5-tropic isolates in DC-PBL co-cultures was linearly associated with the plasma viral RNA levels in a cohort of HIV-1 infected individuals exhibiting an inverse relationship between plasma viral RNA and CD4 cell count. Furthermore, infectivity activity of Nef variants in context of DC-mediated enhanced infection of T-cells also showed a linear relationship to plasma viral RNA levels.

Conclusion—These results illustrate that replication capacity of HIV-1 in DC-T-cell cultures is a significant predictor of plasma viral RNA level. The data suggest that adaptation of HIV-1 to DC interactions with T-cells influences the level of viral replication in the host.

Keywords

HIV-1; Dendritic cells; Replication capacity; CD4; Viral burden; CCR5

Introduction

Viral load has a strong predictive value for human immunodeficiency virus type-1 (HIV-1) disease progression.^{1–3} Studies suggest that viral set points are the result of a complex and dynamic interplay between the infecting virus and the host.^{4, 5} Both host genetic factors (e.g. HLA Class I; chemokine receptors, CCR2, CCR5; chemokines, CCL3L1, CCL4, CCL2) and viral genetic factors have been implicated in influencing the level of plasma viral load and disease progression.^{6–10} Absent from these earlier studies is the evaluation of whether the efficiency of cell-mediated virus transmission may also influence viral load in the host.

*Corresponding Author: Department of Molecular Virology and Microbiology, BCM385, Room 811D, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030 USA, Tel: 713-798-4536, FAX: 713-798-4435, jkimata@bcm.tmc.edu.

Dendritic cells (DCs) have been hypothesized to play a key role in human immunodeficiency virus type-1 (HIV-1) infection and replication.¹¹ Notably, DCs are among the first cell types, along with CD4+ T-cells and macrophages, that encounter HIV-1 following mucosal transmission.¹² HIV-1 exploits the biological function of DCs to facilitate its transport from the site of entry at mucosal tissue to lymphoid tissues where robust replication occurs in CD4+ T-cells. Additionally, DCs efficiently capture and transmit HIV-1 to T-cells via infectious synapses that concentrate HIV and viral receptors.¹³ Moreover, it has also been shown that a cell-contact dependent mechanism of HIV-1 infection is more efficient than cell-free infection, suggesting that cell-cell interactions may be critical for transmission and replication and that contact between DCs and T cell may play a key role in driving HIV-1 replication.^{14, 15} Productive infection of DCs by HIV-1 is not required for enhanced infection as DCs express a number of viral attachment factors including DC-SIGN, syndecans, CD4, and glycosphingolipids that can bind, concentrate, and retain infectious virions for up to 48 hours.¹¹ Together, these data suggest that adaptation of HIV-1 to antigen-presenting cell-T-cell interactions may influence replication and persistence. However, while significant progress has been made toward understanding the mechanisms underlying DC-mediated transmission of HIV-1, whether these interactions contribute to the level of viral replication in the host remains largely unexplored.

In the present study, we sought to determine whether replicative capacity of HIV-1 contributes to the level of plasma viral load. Using HIV-1 variants isolated from chronically infected, antiretroviral treatment (ART) naïve individuals, we investigated the relationship between ex vivo infectivity and replication capacity of CCR5-tropic HIV-1 and plasma viral RNA levels and CD4+ cell counts in the host. In particular, as viral replication mainly occurs in lymphoid tissues of the host and is likely promoted by antigen presenting cell-T-cell interactions,^{14, 16–18} we examined whether the level of HIV-1 infection of T-cells mediated by dendritic cells *in trans* would correlate with plasma viral RNA measurements from the infected individuals. Our data suggest a significant role for viral replicative capacity in determining the level of plasma viral load, and further suggest a role for dendritic cells in driving viral replication in T-cells.

Methods

Human Subjects

A cohort of individuals infected with HIV-1 were recruited from patients seeking treatment at the Thomas Street Health Center, Houston, TX. The subjects had broad range of plasma viral RNA levels (ranging <1,000 to >100,000 RNA copies/ml) and CD4+ cell counts (ranging from 26 to 1027 cell/mm³) (Table 1). All the individuals participating in the study were antiretroviral treatment (ART) naïve at the time of blood draw. Informed consent was obtained from all the participants in this study in accordance with UTHSC-Houston Committee for the Protection of Human Subjects. Buffy coats from healthy anonymous donors were obtained from Gulf Coast Regional Blood Center, Houston, Texas.

Quantification of plasma viral load

Plasma HIV-1 RNA was quantified by using the Amplicor HIV-1 Monitor test, version 1.5 (Roche Diagnostics, Indianapolis, IN) with a detection limit of 400 copies/ml.

Analysis of CD4+ cell count

Immunophenotyping for CD4+ cells was done by flow cytometry. Peripheral blood mononuclear cells (PBMCs) isolated from HIV-1 infected individuals were stained with CD4 monoclonal antibody according to standard procedures for cytometric analysis (BD Biosciences, San Jose, CA).

CCR5 genotyping

Genomic DNA was isolated from the PBMCs of HIV-1 infected individuals or healthy donors using QIAamp DNA Blood Mini kit (Qiagen, Germantown, MD). CCR5 was amplified by PCR and the DNA products were analyzed on ethidium bromide stained 3% agarose gels as previously described.¹⁹ Homozygous wild type and homozygous mutant for delta 32-bp deletion were run in parallel as control.

HIV-1 isolation and titer determination

HIV-1 was isolated from the infected PBMCs by co-culturing with PBMCs from anonymous healthy donors according to standard techniques.²⁰ Donor PBMCs used for HIV-1 isolation and titer determination were first screened for the CCR5 delta 32 mutant allele. Donor samples positive for the mutation were excluded from being used for virus isolation or titer determination. Briefly, PBMCs from HIV-1 infected individuals were diluted at a concentration of 1×10^6 in RPMI 1640 medium supplemented with 20% heat inactivated fetal bovine serum (FBS), 2mM L-glutamine, 100U/ml of penicillin, 0.1µg/ml of Streptomycin constituting complete RPMI and Interleukin-2 (IL-2) (Roche Diagnostics) (50U/ml). PBMCs from infected individuals were then co-cultured with equal number of Phytohemagglutinin (PHA) and IL-2 stimulated healthy donor PBMCs (1×10^6 /ml) in T-25 flask. Every week, culture supernatants were tested for p24 HIV-1 antigen by ELISA kit (Beckman Coulter, Fullerton, CA). At the same time, half of the culture volume was replaced with fresh PHA and IL-2 stimulated donor PBMCs and propagated for three weeks. From these cultures, the cell-free supernatant was stored at -70°C until use. Viral stock titers were determined using healthy donors' PBMCs that were stimulated with PHA and IL-2 (PHA-lymphoblasts) for 3 days as previously described.²⁰ The 50% tissue culture infective dose was calculated by the Spearman-Kärber method.

Characterization of co-receptor usage

GHOST indicator cells were used to determine co-receptor usage of each viral isolate. GHOST-X4 and GHOST-Hi5 cell lines were obtained from AIDS Research and Reference Reagent Program. The cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat inactivated FBS, 2mM L-glutamine, 100 U of penicillin per ml and 100 µg of Streptomycin per ml (DMEM complete). GHOST assays were performed as described earlier.²¹

In-vitro HIV-replication capacity

Human monocyte-derived dendritic cells (DCs) were generated from PBMCs of HIV-negative anonymous blood donors as previously described.²² Briefly, CD14+ monocytes were isolated using anti-CD14+ microbeads and miniMACS systems according to manufacturer instructions (Miltenyi Biotec, Auburn, CA). The CD14+ monocytes were then washed with complete RPMI media and cultured with 1000U/ml of granulocyte-macrophage colony stimulating factor (GM-CSF) and Interleukin-4 (IL-4) (R&D Systems, Minneapolis, MN) in complete RPMI media for 7 days to generate DCs. By FACS analysis, high expression (>95%) of DC-SIGN (CD209) on DCs confirmed the purity of the population.

DCs (1×10^5) were incubated with 2000 TCID₅₀ of different viral isolates in triplicates in a total volume of 200µl in U-bottom 96 well plate and incubated for 3 hrs. The cells were then washed 3 times with RPMI media and co-cultured with 2×10^5 PBLs from the same donor. The cells were co-cultured for 14 days in presence of IL-2 (50U/ml) in complete RPMI. Supernatants were collected every second day and replenished with fresh media and IL-2. Viral replication was assessed by measuring the amount p24 HIV-1 antigen in culture supernatants by ELISA (Beckman Coulter, Fullerton, CA). Replication rates were

determined by calculating the slope (p24pg/ml/day) of the exponential phase p24^{gag} accumulation. All the donor PBMCs were genotyped for delta 32-bp deletion in CCR5 gene. Only donors who were homozygous wild type at the CCR5 locus were used to measure replication rates.

Nef amplification, sequencing and infection assays

Genomic DNA was isolated from HIV-1 infected PBMCs as described above. Variant *nef* alleles from each patient were amplified by nested Polymerase Chain Reaction using primers as described earlier^{23, 24} and cloned into TOPO vector pCR 2.1 (Invitrogen) for sequencing. Sequences were aligned using MacVector (version 10.0.2) and compared to HIV-1_{NL4-3} as a standard sequence. *Nef* alleles were further cloned into the bicistronic expression vector, pIRES2-EGFP (BD Biosciences Clontech, Palo Alto, CA). For expression analyses, cell lysates, prepared after the transfection of Nef-IRES-EGFP variants in 293T cells, were run on SDS-PAGE (Bio Rad, Hercules, CA) and immunoblotted against Nef antisera²⁴ or an anti-GFP monoclonal antibody (Invitrogen). Furthermore, to assess viral infectivity, single cycle infection assays were also carried out in DC capture-transfer assays using HIV-luciferase (HIV-luc) pseudotyped with a R5-tropic envelope (Q168env2²⁵) and *trans* complemented with *Nef* variants. Infectivity was measured by luciferase assay. Incorporation of *Nef* into virions was also assessed by recovering virions from co-transfected cell culture supernatants by centrifugation at $23,600 \times g$ for 1 hr at 4°C and analyzing viral lysates after SDS-PAGE by immunoblotting using Nef antisera and p24 specific antibodies.

Real time PCR for the determination of gene copy number of CCL3L1

Taqman assay for CCL3L1 was similar to that used earlier⁶. Briefly, Real time PCR was done using an ABI 7500 (Applied BioSystems Inc.) with the following universal cycling conditions: 2min at 50°C, 10 min at 95°C and 40 cycles of 15s at 95°C and 1 min at 60°C. All the samples were tested in triplicate. Calculations were based on the estimates of template quantities, after comparing the threshold cycle (Ct) numbers at which positive (over background) fluorescence was detected.

Statistics

Correlations between viral replicative capacity and plasma viral load were examined by linear regression. The relationships between viral replicative capacity or plasma viral RNA loads and CD4+ T-cell counts were analyzed by Poisson regression. Pearson's correlation was used to test for correlation between relative Nef-mediated enhancement of infection activity and plasma viral load. Correlation between plasma viral load and copy number of CCL3L1 was tested using Spearman's correlation. Statistical analyses were performed using STATA 10 software.

Results

Study Subjects

HIV-1 isolation was attempted from PBMC specimens collected from 20 chronically HIV-1 infected individuals prior to antiretroviral treatment. Characteristics of the HIV-1 infected individuals are presented in Table 1. Subjects were selected, irrespective of disease status, and included individuals with a wide range of plasma viral RNA levels. The group included five females and 15 males of mean age 37.8 ± 4.9 (range, 33 to 46) and 38.13 ± 4.8 (range, 19 to 50), respectively. We were not able to recover infectious virus from PBMCs of two patients (#7 and #365). The median plasma HIV-1 RNA level was 84,252.5 copies/ml (range, 428 to 750,000 copies/ml) and, the median CD4+ cell count was 316 cells/mm³

(range, 26 to 1027 cells/mm³). The HIV-1 infected individuals included in this study showed significant inverse relationship between the between the plasma viral load and CD4+ cell counts ($p = 0.02$; 95% CI $-0.512, -0.044$) (Fig. 1).

Co-receptor Specificity of viral isolates

To determine the coreceptor usage of the viral isolates, we used the GHOST indicator cell lines expressing either the CCR5 or CXCR4 coreceptors. HIV-1 clones, Yu2 and NL4-3, were used as controls for R5 and X4 dependent infections, respectively. GFP expression was detected in GHOST-Hi5 cells in case of infection by all the viral isolates, indicating that all the viral isolates were R5 tropic except for isolate JA, which was R5X4 dual-tropic by this assay (Table 1).

Relationship between HIV-1 replication capacity and plasma viral RNA concentration

Initially, we examined the replicative capacity of the viral isolates in PHA-lymphoblasts. However, several of the viral isolates replicated poorly or inconsistently, prompting us to consider an alternative cell culture system to evaluate viral replicative capacity (data not shown). We therefore measured the replication rate of viral isolates by performing p24 HIV-1 antigen ELISA on supernatants from DC capture-transfer assays. Importantly, viral replication has been shown to be most robust in these culture conditions and DCs may enhance infection and replication in the host.^{11, 14, 15, 26–28} Viral replication was compared using cells derived from the same donor. The replication rates were determined from the slope of the exponential phase of p24 production. Out of 18 viral isolates, two did not show any detectable p24 antigen by ELISA. The median of replication rate was 0.2763 log₁₀pg/ml/day (range, 0.0653–0.7389 log₁₀pg/ml/day). There was a significant linear relationship ($r^2 = 0.283$, $p = 0.04$) between the plasma viral load and HIV-1 replication capacity (Fig. 2A), indicating that replication capacity of the virus may significantly influence plasma viral load in the host. Additionally, HIV-1 replication of selected viral isolates was determined in DC-PBL co-cultures using cells from a different donor in order to compare the inter-individual variability. Comparable replication rates were observed with the second donor (Table 2).

HIV-1 replication Capacity and CD4+ T cell count

To determine if there was also a relationship between RC values and CD4+ T-cell counts. Correlation between replication rates of the viral isolates and CD4+ T cell count in the host was statistically determined by Poisson regression analysis. The data demonstrate a decreasing trend for CD4+ T-cell counts with an increase in replication rate (Fig. 2B), although it was not statistically significant ($p = 0.09$; 95% CI $-3.02, 0.23$).

Plasma HIV-1 viral load and viral genetic determinant

Because Nef function significantly affects viral replication, we examined whether mutations in Nef affecting viral infectivity accounted for the differences in viral load or replicative capacity in DC-mediated *in trans* infection of T-cells. We amplified and sequenced the predominant *nef* variants from the HIV-1 infected patients and confirmed their expression in 293T cells by immunoblot (Fig. 3A). No *null* Nef mutants were identified and Nef protein was detected from each allele. Furthermore, to examine the effect of Nef on viral infectivity in DC capture-transfer assays, a *nef* deleted HIV-1 clone containing a luciferase reporter was complemented with each of the Nef variants *in trans* and a CCR5-tropic Env. HIV-1_{NL4-3} *nef* and a construct with no *nef* gene served as positive and negative controls, respectively and comparisons were made with the activity of the HIV-1_{NL4-3} *nef* allele to calculate a relative viral infectivity value. Interestingly, incorporation of Nef in virions was only observed in 6 of the 15 viruses (Fig. 3B). Furthermore, there was no significant difference

($p=0.89$) between the average infectivity of the Nef-negative viruses (1.45 ± 0.26 [mean + standard error of the mean]) and Nef-positive viruses (1.51 ± 0.40), suggesting that virion-association of Nef does not enhance viral infectivity. However, we found a significant linear association ($r = 0.5362$; $p = 0.039$) between relative Nef activity and viral load (Fig. 3C), indicating a significant influence of Nef enhancement of infectivity on viral load in the host. There was also a weak, but not significant, association observed between Nef activity and *in vitro* replicative capacity of viral isolates (data not shown). These results were confirmed in cells derived from two additional donors.

Plasma HIV-1 viral load and host genetic determinants

Because all of the viral isolates tested were CCR5-tropic, we examine the virus donors for the 32 bp deletion in CCR5 gene and copy number of CCL3L1 genes to exclude the possibility that genetic determinants in these genes accounted for the differences in viral loads. By PCR analysis, we did not observe any CCR5-delta32 homozygous mutant. All the HIV-1 infected individuals were homozygous wild type for CCR5 locus except subject #JA (Table 1). Next, we examined the gene copy number of CCL3L1 in HIV-1 infected individuals by quantitative Real Time PCR (Table 1). No correlation between the plasma viral RNA levels and copy number of CCL3L1 gene in HIV-1 infected individuals was observed ($p > 0.05$, nonparametric Spearman's Correlation).

Discussion

HIV-1 disease progression has been correlated with plasma viral RNA levels¹⁻³, although the viral and host determinants contributing to the level of virus are incompletely understood. Early studies demonstrated phenotypic changes in the infecting virus were associated with progression to AIDS, particularly the appearance of cytopathic variants that replicated efficiently in CD4+ T-cells.⁹ Indeed, our studies with the SIV-macaque model demonstrated that the appearance of variants with greater replicative capacity in CD4+ T-cells influenced plasma viral load and the rate of disease progression.²⁹ Importantly, highly pathogenic variants also show greater replicative capacity in DC-T-cell cocultures compared to the minimally pathogenic parental virus^{26, 30}, suggesting that SIV, and by extension HIV-1, evolve during infection to exploit DC-T-cell interactions for viral replication. The data presented here support this hypothesis and show a direct relationship between plasma viral RNA levels in the host and infectivity and replication capacity of the infecting viruses in DC-T-cell cocultures.

We conducted a study with 20 HIV-1 infected individuals who were antiretroviral naive at the time of virus isolation to evaluate the replication rate of HIV-1 viral isolates *in vitro* from the patients with low or high viral load irrespective of disease status. Several approaches have been used to measure *in vitro* HIV-1 replication capacity.³¹ Most commonly, replication rate has been studied by measuring the p24 HIV-1 antigen in supernatants after infecting the PHA-lymphoblasts from healthy donors. Because several of the viruses we isolated replicate poorly in PHA-lymphoblasts, we performed virion capture and transfer assays with DCs and T-cells to study the replication rate of R5-tropic viral isolates. In particular, a DC-mediated capture-transfer assay was used since HIV-1 infection of CD4+ T-cells has been shown to be most efficient when virions are presented in association with macrophages or DCs.^{11, 14, 15, 26-28} We observed a significant linear relationship between HIV-1 replication rate in DC-PBL cocultures and plasma HIV-1 RNA concentration in the host. Thus, these data suggest that replication capacity of the virus is a major determinant of plasma viral load, and hence, disease progression. Furthermore, variants that efficiently utilize antigen presenting cell-T-cell interactions may have a replicative advantage in the host. Variation in transmission efficiency of different isolates may also reflect upon replication capacity, but transmission differences could be in part due

to the levels of expression of DC-SIGN,³² DC-SIGN-mediated infectious synapse formation³³ or level of CD4 co-expression.³⁴

Since our analysis remained limited to R5-tropic HIV-1, we evaluated the role of genetic determinants of the virus contributing to plasma viral load other than those in envelope that alter co-receptor usage. Viruses carrying deleterious mutations in the *nef* gene have been shown to be less fit than the wild type viruses.³⁵ Furthermore, the slow replicating viruses associated with a low viral load in case of long-term survivors has been reported to harbor defect in the *nef* gene. Thus, we PCR amplified and sequenced Nef variants and performed single cycle infection assays in DC-PBL co-cultures in order to assess Nef-mediated viral infectivity. There was significant linear association observed between the viral infectivity enhancement by the predominant Nef variants in DC-mediated *in trans* infection of T-cells and viral RNA levels in the plasma of the hosts from which the *nef* alleles were cloned, further demonstrating the importance of Nef-mediated enhancement of infectivity on viral replication *in vivo*.^{35–37} Interestingly, although Nef protein was expressed from all *nef* alleles, only a third of the Nef proteins were found to be virion associated. Moreover, there was no correlation between virion incorporation of Nef and enhanced viral infectivity. These data are consistent with recent studies indicating that Nef affects virion infectivity during virion biogenesis in the infected cell, and not because of incorporation into virions.^{38–40} Finally, although our study group showed strong correlation between replicative capacity and viral RNA levels in plasma, we observed little association between Nef activity and replicative capacity, indicating that there may be other viral factors influencing RC either independent or in conjunction with Nef-mediated enhancement of infectivity.

Since all the viral isolates were R5-tropic, we examined whether CCR5 gene deletion (CCR5Δ32) or gene copy number of CCL3L1 was influencing plasma viral load. Both CCR5 and CCL3L1 have been shown to have a strong influence on the disease progression.^{6, 10} All the individuals studied in our study were wild type homozygous for CCR5 gene, except for one who was heterozygous. There was no significant association between plasma viral load and CCL3L1 copy number. Although recent findings implicated that the individuals with lower than the average number of CCL3L1 genes for their ethnic group were found to be more susceptible to HIV-1 infection in contrast to the ones having more than the average number of CCL3L1 copies, large cohorts were required to observe this effect.^{6, 41} Contrary to this and in support of our observation, Shao et al.,⁴² recently reported that copy number variations in CCL3L1 failed to exert major impact on the outcome after HIV-1 infection in smaller adolescent population. In view of these observations, our data suggest that replication capacity of HIV-1 is a significant determinant of viral load. What remains unclear are the cellular factors affecting replication capacity of the virus.

In conclusion, our study shows a linear correlation between replication capacity of HIV-1 variants with plasma viral load. Because this was observed in the context of DC-PBL co-cultures, it supports our earlier observations with the SIV-macaque model that the efficiency of replication in response to DC-T-cell interactions influences plasma viral load. Thus, adaptation of HIV-1 to antigen presenting cell-T-cell interactions during the course of infection may drive viral replication and disease progression.

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Fig. 1. Association between plasma viral load and CD4 counts

Plasma HIV-1 RNA levels versus CD4+ cell counts determined in chronically infected, ART naïve subjects at the time of blood specimen collection are shown. Poisson regression analysis indicates a significant inverse relationship between plasma viral load and CD4+ cell counts ($p=0.02$; 95% CI $-0.512, -0.044$).

Fig. 2. Correlation of in vitro replication capacity with plasma HIV-1 viral load and CD4 counts (A) HIV-1 replication assays were performed in DC-PBL co-cultures and the rate of p24 antigen production was determined during the logarithmic phase of viral replication. Solid line indicates a strong linear relationship between replication capacity and plasma viral load by linear regression ($r^2 = 0.283$; $p = 0.04$). (B) CD4+ cell counts in the peripheral blood and replication rates determined in DC-PBL co-cultures are given in Table 1. Solid lines illustrate a modest inverse trend ($p = 0.09$; 95% CI -3.02, 0.23) between CD4+ cell counts and replication rate.

Fig. 3. Relationship between nef mediated enhancement of viral infectivity and plasma viral load (A) Expression of Nef variants alleles cloned into the pIRES2-EGFP bicistronic vector. Cell lysates were separated by SDS-PAGE and immunoblotted against Nef specific anti-sera and anti-GFP antibody. Shown are the Nef variants compared to vectors with and without HIV-1_{NL4-3} Nef. (B) Detection of virion-associated Nef. Virion lysates were separated by SDS-PAGE and immunoblotted against Nef specific anti-sera and p24 specific antibody. (C) Nef-mediated viral infectivity. Single cycle infection assays were carried out in DC-PBL co-cultures using HIV-luciferase (HIV-luc) pseudotyped virus and infectivity was measured by luciferase assay. Pearson's correlation demonstrates a statistically significant linear association (solid line) between relative Nef activity and plasma viral load ($r = 0.5362$; $p = 0.039$).

Table 1

Characteristics of chronically infected HIV-1 patients

Patient ID	Viral Load (RNA copies/ml)	No. of CD4 Cells/mm ³	CCR5 Status ^a	CCL3L1 (copies/genome)	Co-receptor usage ^b
#1	428	1027	Wt	2	R5
#3	780	591	Wt	7	R5
#365	1,140	604	Wt	6	ND
#560	2,252	414	Wt	3	R5
#JF	6,090	305	Wt	5	R5
#A	7,410	419	Wt	3	R5
#MS	9,384	322	Wt	2	R5
#DC	32,748	193	Wt	4	R5
#532	74,066	700	Wt	2	R5
#JA	79,289	80	Wt	1	R5X4
#B	89,216	432	Wt	3	R5
#4	121,179	235	Wt	5	R5
#508	134,073	225	Wt	3	R5
#7	203,592	190	Wt	2	ND
#740	230,011	26	Wt	6	R5
#2	253,418	536	Wt	3	R5
#359	502,088	273	Wt	8	R5
#873	712,734	504	Wt	3	R5
#656	750,000	310	Wt	7	R5
#5	750,000	116	Het	2	R5

^a CCR5 status was determined by PCR amplification. Wt, wild type CCR5 and Het, Heterozygous for delta 32 mutant allele.

^b Coreceptor usage was determined using the GHOST cell assay. R5, R5 tropic viral isolates; R5X4, dual tropic viral isolates; ND, not determined because virus could not be isolated.

Table 2

Comparison of HIV-1 replication capacity in PBMCs from different donors.

Patient ID	Viral replication (log ₁₀ p24pg/ml/day)	
	Donor1	Donor2
#2	0.361	0.354
#3	0.276	ND
#4	0.455	0.447
#JF	0.258	0.222

ND, no virus was detected by p24 antigen ELISA in replication assays.