Expression of Unique and Diverse HIV Variants in Cerebrospinal Fluid during ART

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Background: The central nervous system (CNS) may become a sanctuary site for HIV during stable antiretroviral therapy (ART). The determinants and mechanisms of viral persistence in CNS remain poorly understood, and conflicting data create a limited understanding of the associations between virus detection in plasma and virus detection in the CNS.

We investigated the possible cellular sources of HIV persisting in cerebrospinal fluid (CSF) under ART using a particle immunocapture algorithm targeting source cell proteins embedded within HIV envelopes and examined the phylogenetic relatedness of plasma and CSF viruses.

Methods: From the PARTITION Study[1] of persons with persistent CNS viremia under HAART, we examined 6 HIV-positive persons on stable ART who underwent lumbar puncture for neurological disease (n=4) or a history of intermittent plasma viremia (n=2). Virions expressed in CSF were segregated by source cell type by targeting 10 different host cell proteins that may embed in HIV envelopes during budding. Virions from each capture step were sequenced for evidence of reverse transcriptase (RT) drug-resistant variants (DrVs), and for relatedness to other viruses in the CSF and in plasma. Drug concentrations in plasma and CSF were measured by HPLC.

Aims: 1) To genetically characterize HIV variants persisting in CSF and the relationship of DrVs to current and previous ARV regimens,

2) To examine if variants in the CSF were homogeneous to HIV detectable in blood plasma,

3) To identify the possible cellular sources of variant populations in the CSF by targeting host cell-type proteins embedded in the virion envelopes.

Study Participants

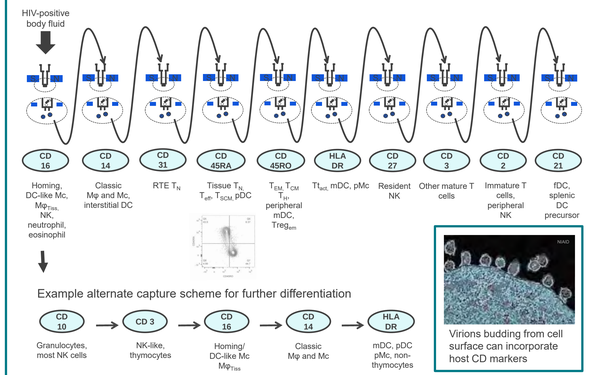
- 6/8 CSF specimens from HIV-infected persons with extensive treatment histories yielded recoverable virus
- Only 2/8 persons with CSF viremia had marked plasma viremia
- No evidence of malignancies in the participants

Table. Characteristics at time of paired plasma and CSF sampling and HIV RT drug resistance mutations (DRMs) from the PARTITION participants

Participant ID		01A	01B	04A	05B	10A	14A
ART regimen		TDF FTC MVC RAL	TDF FTC DRV/r	AZT 3TC EFV	ABC 3TC DRV/r	TDF FTC RAL	3TC MVC RAL DRV/r
Other (prior) ARVs		None	3TC ABC	None	AZT SQV/r LPV/r	None	ABC AZT
CPE score		10	7		8	7	6
Plasma/CSF drug concentration ng/mL		TDF 146/- FTC 1984/- RAL 123/18	TDF -/38 FTC -/63 DRV 1196/20	AZT 298/103 3TC 866/182 EFV 996/22	3TC 35/- DRV 1933/25	RAL <LLQ/<LLQ	MVC 118/48 RAL 1237/171 DRV2667/177
CD4 cells/mm^3		185	660	449	734	342	374
Nadir CD4 cells/mm^3		-	44	390	323	-	30
Plasma/CSF VL c/mL		52/1231	88/1569	3443/13088	258/3518	10817/63010	<40/1981
DRMs	CSF	K103N [mφ, $_m$NK]	M184I ($_h$Mc: None)	K103N [NK], V108I, E138A	L74V, M184V	None	K65E [T_H(17)], V108I, V179D [T_N], M184V
	Plasma current	-	-	None	M184V	None	-
	Plasma historic	-	K103R M184I	None	-	-	-

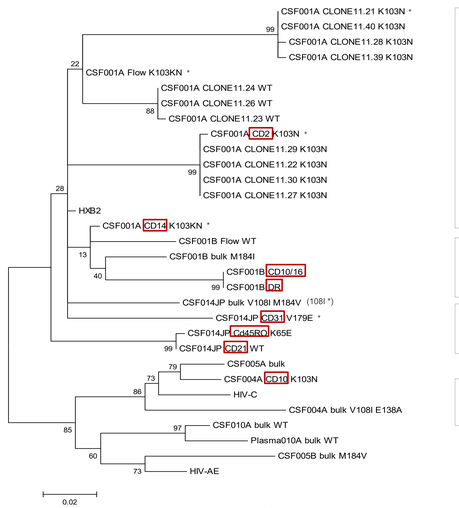
-, not available; CPE, CNS penetration effectiveness; <LLQ, below the 10ng/ml limit of quantitation by HPLC-MS/MS; [ ], cell source suggested by capture: mφ, macrophage; $_m$NK, mature natural killer cells; $_h$Mc, homing monocyte; T_N, naïve thymocyte

HIV Particle Immunocapture Algorithm



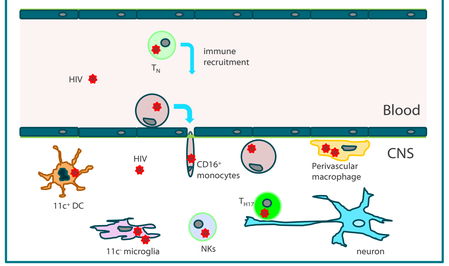
Capture algorithm showing sequence of antibody targets designed to isolate virus particles originating from different immune cells. DC, dendritic cell; Mc, monocyte; MφTiss, tissue resident macrophage; NK, natural killer cell; RTE, recent thymic emigrant; T_N, naïve thymocyte; $T_{EM, CM, TM}$, effector, central, transitional memory; T_H, T helper, $T_{act,reg}$, activated, regulatory thymocyte; mDC/pDC, myeloid/plasmacytoid dendritic cell; FDC, follicular dendritic cell.

Sequence analysis. Viral RNAs obtained from capture steps were RT-PCR amplified and sequenced by the Sanger method (ABI 3130XL). For specimens with sufficient template, clonal analysis was performed for comparison to capture sequences.



Phylogenetic tree of HIV sequences obtained from the CSF and plasma of participants. Bulk and capture-specific sequences were used for this analysis. Identified drug resistance mutations are indicated. *, no record of NNRTI exposure. T93+G4 analysis with 1000 bootstrap replicates in MEGA 5.2.

Key cellular participants in CNS HIV expression



Results

At sampling, HIV-1 RNA levels in CSF were median 1.2 $\log_{10}$ c/ml (range 0.4-2.0) higher than in paired plasma (Table). ARV drug concentrations were lower in the CSF relative to blood, particularly for DRV, RAL and EFV. The captures identified distinct HIV variants in the CSF of 4/6 individuals.

ID **04A**, on an efavirenz (EFV)-containing regimen expressed three different NNRTI-resistant variants in the CSF associated with RA$^-$/RO$^-$/HLADR$^-$/CD3$^-$/CD2$^+$ and/or CD10$^+$ particles. These marker phenotypes are typically associated with NK cells. There was no evidence of plasma DrVs.

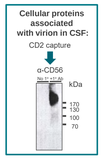
ID **01B**, with prior abacavir (ABC)/lamivudine (3TC) and current emtricitabine (FTC) exposure, expressed majority M184I resistance in CSF. CSF HIV of this participant was identified from captures against CD10, CD16 and HLADR.

ID **14A**, with prior ABC exposure, had a K65E variant associated with CD16$^-$/CD14$^-$/CD45RA$^-$/CD45RO$^+$, suggesting T_H(17) cells as a possible source.

ID **10A,** with unsuppressed viremia, had phylogenetically distant wildtype viruses in the plasma and CSF, and ART drugs were undetectable.

Two subjects (ID 01A and 14A) with no reported NNRTI exposure expressed NNRTI-resistant variants in CSF, while plasma viremia was suppressed.

ID **05B**, with current and past exposures to 3TC, had M184V DrVs in both the blood and CSF.



Example Western blot analysis of cellular CD proteins bound by the capture process from the CSF of an HIV-infected person. This analysis verified that expected full-length (transmembrane) proteins were being bound.
For this participant (01A), a DrV was first identified from the anti-CD2 step in the algorithm. Additional analysis found that the virus also precipitated with anti-CD56. This suggested mature NK cells as the source. Lanes are with and without anti-CD56 Ab.

Summary

- Multiple DrVs and different subpopulations carrying the same resistance mutation were identified in the CSF of individual participants
- DRMs that were not identified in the bulk CSF sequences were found in minority DrVs in the CSF anti-CD captures, and associated with markers for classical Mc/macrophages, homing Mc, NK and T_H cells
- In the majority of cases, the drug resistance mutations that were present in the CSF capture sequences did not represent mutations associated with the current treatment regimen, except in ID 04A (CD10 K103N – resistance to EFV) and ID 14A (CD31 V179E –low level NNRTI resistance and CD45RO K65E –low level resistance to ABC)
- Virus from the plasma of two individuals showed only wild type and was suppressed in two other persons, thus blood compartment variants did not explain the presence of the DrVs found in the CSF
- There was a substantial amount of virus identified from the anti-CD2 captures, which in the capture scheme may represent a natural killer (NK) cell source
- Absence of malignancy excludes confounding interpretation of cellular markers (CD2/10/21) that are also associated with these conditions
- EFV and DRV concentrations in CSF were as low as 1-2% of that detected in plasma
- Low-level variants in plasma were more homogeneous than in CSF

Conclusions

- HIV populations persistently expressed in the CNS during ART were genetically diverse, consisting of both WT and drug-resistant viruses
- Minority DrVs from prior ART history maintained expression in CSF
- We found CSF HIV variants that were distinct from plasma viruses, suggesting CNS HIV evolution and maintenance that is separate from blood
- Lower CNS drug concentrations and/or activity may allow for compartmentalized selection, persistence and evolution of DrVs and insufficient activity against WT viruses
- The capture data suggest different resident cell types were lending to ongoing CNS HIV expression under ART, underscoring a lack of suppression in a variety of cellular reservoirs

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1. Nightingale S, Geretti AM, Beloukas A, et al. Discordant CSF/plasma HIV-1 RNA in patients with unexplained low-level viraemia. *J Neurovirol.* 2016; 22:852-860

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