HIV-1 Replication in the CNS Is Associated With Increased Neurocognitive Impairment

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Abstract

Background: HIV-infected individuals are reported to have higher levels of neurocognitive impairment than HIV-negative controls. We examined the association between independent HIV-1 replication in the CNS and neurocognitive impairment.

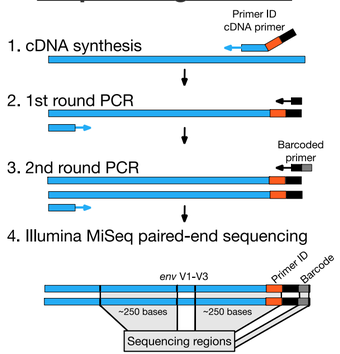
Methods: We used cerebrospinal fluid (CSF) and blood plasma samples from 40 subjects off antiretroviral therapy (ART) to characterize viral populations in the CNS and blood. Subjects were enrolled in 1 of 4 cohorts. Two cohorts enrolled subjects based on neurological symptoms and two enrolled subjects based on CD4 count and viral load. All subjects had neurocognitive assessments. cDNA was generated from viral RNA and partial *env* genes were amplified and sequenced by Illumina deep sequencing with Primer ID to quantify the number of templates examined (Fig. 1A); and/or full length *env* genes were amplified and sequenced by single genome amplification (SGA)(Fig. 1B). Pseudotyped viruses were generated from cloned, full-length *env* genes and used in entry assays to determine whether Env proteins were well-adapted to entering cells expressing low levels of CD4 (i.e. macrophage-tropic; Fig 1C).

Results: Based on formal neurocognitive assessments, subjects were classified into three groups with increasing levels of neurocognitive impairment: 1) normal (N=7), 2) mild neurocognitive disorder or asymptomatic neurocognitive impairment (N=10), and 3) HIV-associated dementia (N=23). The observation of genetically distinct, viral lineages in the CSF was evidence of ongoing replication in that compartment, and these lineages were considered compartmentalized (Fig. 1A and 1B). Increasing neurocognitive impairment was positively associated with compartmentalization (exact trend test, p=0.04). For 71% of subjects with compartmentalized HIV-1 in their CSF, this virus was also macrophage-tropic, and thus compartmentalization was significantly associated with macrophage tropism (fisher's exact test, p= 0.001).

Conclusions: HIV-1 replication in the CNS is strongly associated with neurocognitive impairment and the majority of subjects with evidence of ongoing HIV-1 replication in the CNS have macrophage-tropic HIV-1 in that compartment. These results suggest that there may be a causal relationship between HIV-1 replication in macrophage-lineage cells in the CNS and neurocognitive symptoms in HIV-infected subjects off therapy. Antiretroviral therapy that fully suppresses viral replication in the CNS, particularly in macrophages, may be more likely to improve neurocognitive performance.

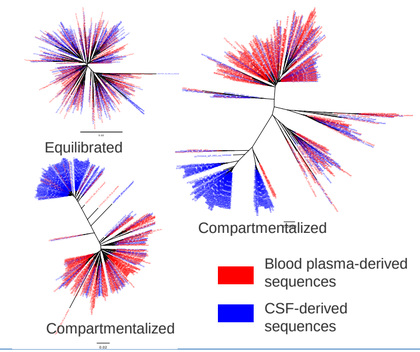
Compartmentalized Lineages in the CSF Are Evidence of Ongoing Viral Replication in That Compartment and Are Associated With Neurocognitive Impairment

Illumina MiSeq 300 bp Paired-end Deep Sequencing Method

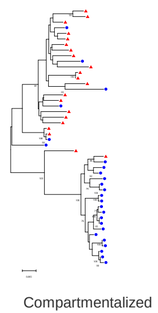


Phylogenetic Analyses to Identify CNS-compartmentalized Viral Lineages

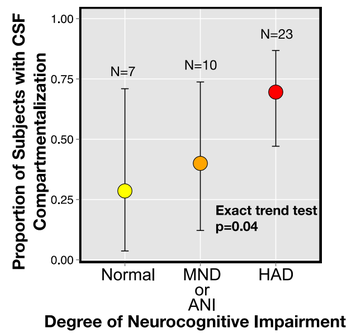
Deep Sequencing of Partial *env* Genes



SGA of Full-length *env* Genes

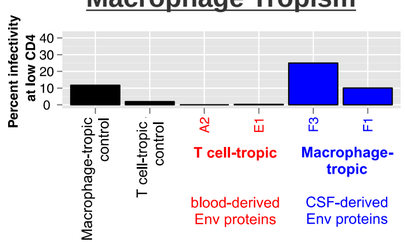


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HIV-1 Replication in the CNS Is Associated With Macrophage-tropism

Affinofile Assay for Macrophage Tropism



Compartmentalization is Significantly Associated with Macrophage-tropism

	Mac-tropic Lineages in the CSF	No Mac-tropic Lineages in the CSF
Compart. Lineages in the CSF	10	4
No Compart. Lineages in the CSF	1	13

Fisher's Exact Test, p= 0.001

Summary

- **CSF-compartmentalization can be used to identify subjects with sustained viral replication in their CNS.**
- **HIV-1 replication in the CNS is positively associated with increasing levels of neurocognitive impairment.**
- **HIV-1 replication in the CNS is significantly associated with macrophage-tropism.**
- **Improve neurocognitive performance in HIV-positive subjects may require fully suppressing viral replication in the CNS, particularly in macrophages.**

Cohorts

40 HIV-positive, Treatment-naïve Subjects Were Enrolled in Four Cohorts

THINC study (based at UNC, UCSF and Yale), USCF (collected by Dr. Richard W. Price and Dr. Serena Spudich), National NeuroAIDS Tissue Consortium (NNTC) and Emory (Collected by Dr. Albert M.L. Anderson). Two of the cohorts (NNTC and Emory) targeted and enrolled subjects with neurocognitive impairment.

CSF and blood plasma samples were collected from each subject and all subjects were given a neurocognitive assessment to determine whether they were neurocognitively impaired.

Cohorts	N	Mean Log10 Blood VL (Q1-Q3)	Mean Log10 CSF VL (Q1-Q3)	Mean CD4 count	Enrolled based on neurocog symptoms	Sequencing method
THINC	15	4.83 (4.50–5.08)	4.01 (3.40–4.40)	254	No	SGA and Deep
UCSF	11	4.78 (4.66–5.14)	4.69 (4.38–5.12)	190	No	SGA
NNTC	5	5.27 (4.73-5.78)	4.48 (3.44–5.88)	62	Yes	SGA and Deep
Emory	9	4.63	3.79 (3.06–	125	Yes	Deep

Acknowledgements

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