Poster # 364

CSF HIV-1 compartmentalization by *env* deep sequencing: relation to neuronal injury

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Introduction

HIV-1 infection of the central nervous system (CNS) is a constant feature of systemic infection, and HIV RNA is detectable in cerebrospinal fluid (CSF) of virtually all untreated viremic individuals. CSF HIV can derive from circulating blood but also from locally replicating populations (compartmentalized) due to either clonal expansion or sustained replication and evolution of diverse populations.

We used partial HIV *env* deep sequencing to survey matched CSF and blood from a convenience sample of untreated individuals grouped by blood CD4+ T cell counts, neurological diagnosis, and concentrations of CSF neurofilament light chain (NFL), a sensitive biomarker of neuronal injury.

Our main ***hypotheses*** underlying this study were:

- *Compartmentalized infection that reflects local (CNS) HIV-1 replication and evolution of diverse HIV-1 sequences is an important factor in the genesis of virus-induced CNS injury, in both frank HIV encephalitis and in less-inflammatory CNS injury.*
- *This will be reflected in detection of compartmentalized HIV-1 infection in CSF samples in individuals who 1) present with clinically overt HAD (and elevated CSF NFL) and those with low blood CD4+ T cell counts and 2) suffer less overt CNS disease documented by elevated CSF NFL concentrations.*
- *Hence, overall, CSF HIV-1 compartmentalization will correlate with neuronal injury as measured by elevated CSF NFL.*

Methods

Study Design. We cross-sectionally examined 43 archived CSF and blood sample pairs from untreated individuals from Gothenburg and San Francisco, and successfully generated sequences from 33 pairs. For this initial survey, individuals were divided into 4 groups based on blood CD4, neurological presentation and age-adjusted CSF NFL concentration as previously described (Price RW et al, *J Neuroimmune Pharm* 2013;8(5):1147-58, PMCID: 3889225; Peterson J, et al, *PloS One* 2014;9(12):e116081. PMCID: 4277428) (Table 1):

1. Neuroasymptomatic (NA) ***CD4 >200*** cells/μL (N=8)
2. NA CD4 <200 with normal CSF NFL (***NFL-negative***) (N=8)
3. NA CD4 <200 with elevated NFL (***NFL+***) (N=10)
4. Clinically diagnosed ***HAD*** (N=7), all with elevated CSF NFL

Virology. Viral RNA from CSF and blood was used to generate cDNA, and the V1/V3 region of env amplified and sequenced using Illumina MiSeq deep sequencing with PrimerID (Zhou Set al. J Virol. 2015;89(16):8540-55. PMC4524263). Sequences corresponding to the same viral genome were used to generate a consensus sequence, and subsequent analyses were based on these consensus sequences. This procedure removed PCR-induced mutations from our analyses and allowed us to accurately assess the frequency of each sequence within the population. Neighbor-joining phylogenetic trees were constructed for each subject and visually segregated into 4 classes (Figure 1):

A. fully **equilibrated** CSF and plasma populations.
B. CSF **clonal expansions** with limited diversity of CSF env genes.
C. **'Minor' diversity**, with genetically diverse compartmentalized CSF populations comprising <30% of CSF env genes.
D. **'Major' diversity** with >30% genetically diverse compartmentalized CSF populations.

Table 1. Subject characteristics

	Site (SF:GOT) ratio	Gender (M:F) ratio	Age (years)	CD4 (cells/μL)	CSF NFL (log10 ng/L)	Plasma HIV RNA (log10 copies/mL)	CSF HIV RNA (log10 copies/mL)	ΔPlasma:CSF HIV RNA (log10 copies/mL)	CSF WBC (per μL)	CSF neopterin (nMol/L)	CSF: blood Albumin ratio
			Mean (SD)			Mean (SD)			Median (IQR ranges)		Mean (SD)
NA, CD4 >200	8:0	8:0	45.2+/-7.4	390.6+/-153.8	2.84+/-0.29	4.38+/-0.49	3.55+/-0.56	0.83+/-0.68	6 (2.8-11.3)	19.0+/-14.5	6.1+/-2.5
NFL-negative	4:4	5:3	38.0+/-8.4	97.9+/-55.7	2.78+/-0.14	4.87+/-0.36	3.86+/-0.50	0.95+/-0.74	6 (3.0-9.0)	32.6+/-20.0	4.8+/-1.9
NFL +	3:7	10:0	43.2+/-8.8	77.4+/-62.0	3.48+/-0.54	5.18+/-0.58	4.01+/-0.78	1.17+/-0.89	2 (0-12.5)	36.0+/-33.6	7.1+/-3.7
HAD	4:3	7:0	43.3+/-10.5	122.0+/-89.4	4.59+/-0.33	5.46+/-0.44	4.91+/-0.47	0.55+/-0.29	13 (2-20)	70.2+/-31.0	12.1+/-9.8

Figure 1. Algorithm for visual classification of CSF:plasma env relationships.

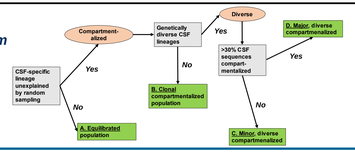
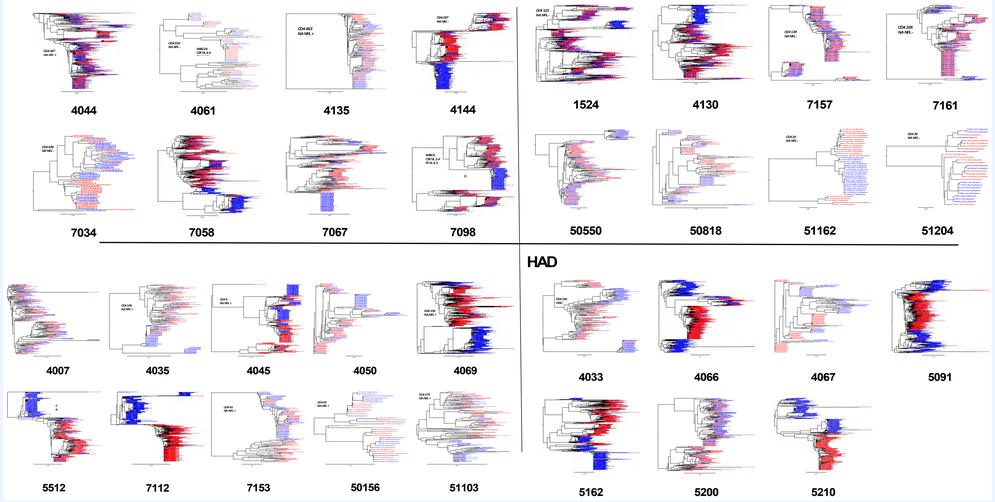


Table 2. CSF:blood HIV Population Relationships

Subject Group	N	Equilibrated A	Clonal expansions B	Minor diverse compartment C	Major diverse compartment D
1. >200 CD4	*8*			37.5%	62.5%
2. NA <200 CD4 NFL-	*8*	12.5%		50.0%	25.0%
3. NA <200 CD4 NFL+	*10*		10.0%	30.0%	60.0%
4. HAD	*7*				100.0%

Figure 2. Phylogenetic analysis of CSF (blue) and plasma (red) HIV-1 env sequences.



Results

The phylogenetic trees comparing the CSF (blue) and plasma (red) *env* sequences from each of the 33 subjects, grouped in the *a priori* clinical categories based on CD4, neurological presentation and CSF NFL, are shown in Figure 2. Table 2 provides a summary of the analysis of compartmentalization using the classification defined in Figure 1.

Summary & Conclusions

CSF HIV-1 *env* compartmentalization is very common in chronic untreated infection.

- Major (>30%) CSF *env* sequence compartmentalization was a universal finding in HAD, noted in all of the 7 HAD subjects in this study.
- However, CSF *env* sequence compartmentalization was also present in the other three subject groups, including the two without evidence of ongoing CNS injury (normal levels of CSF NFL).
- Possibly the character of *env* compartmentalization differed in these other groups, but even if less common and generally less marked in non-neuropathic infection, some cases without evidence of axonal injury were similar to those of HAD and NFL+ groups.

Hence, using the simple definitions of compartmentalization employed here, the results ***do not support our initial hypothesis***.

- These simple measures and definitions of CSF compartmentalization may not distinguish neuropathic HIV-1 infection from 'more innocent' leptomeningeal infection.
- Using these simple measures and definitions, CSF HIV-1 compartmentalization does not provide a simple biomarker of neuropathic infection.

These results indicate that characteristics of CNS infection other than that of simple compartmentalization detected in CSF are important in the pathogenesis of CNS axonal injury. Further work should focus on additional pathogenic factors, including:

- The biological properties of evolving CNS HIV-1 populations.
- CSF immune and inflammatory profiles and their components.

More quantitative and discriminating analytical methods comparing CSF and plasma sequences may also provide greater insight into the role of compartmentalized infection in neuropathogenesis.

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