The effect of cryptococcal meningitis on the CNS reservoir

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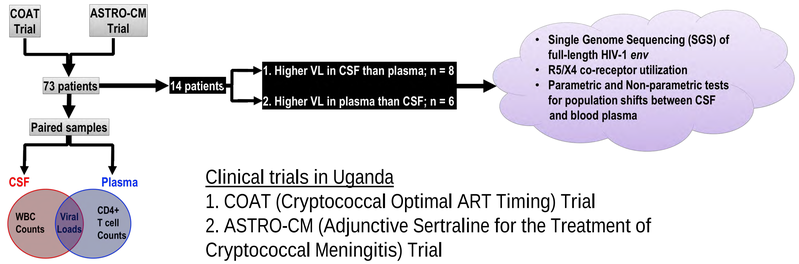
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Background and Study Rationale

Opportunistic central nervous system (CNS) infections are postulated to influence formation and persistence of a HIV CNS reservoir through trafficking of infected activated cells.[1-3] Viral compartmentalization present at initiation of antiretroviral (ART) initiation may affect the establishment of a reservoir. We investigated the role CNS infections have in seeding the HIV reservoir by characterizing viral populations and compartmentalization of virus in blood and cerebrospinal fluid (CSF).

Study Design & Methods



Clinical trials in Uganda
1. COAT (Cryptococcal Optimal ART Timing) Trial
2. ASTRO-CM (Adjunctive Sertraline for the Treatment of Cryptococcal Meningitis) Trial

Results

Table 1: Characteristics of Study Participants at baseline (pre-ART).

At Time of Cryptococcal Meningitis Diagnosis				
	Median [IQR] or N (%)			
n	73			
Age	37 [29, 43]			
Male Sex	34 (68.0%)			
CD4 count, cells/μL	21 [10, 70]			
CSF specimen prior to ART initiation				
CSF WBC, cells/μL	<5 [<5, 25]			
CSF WBC < 5 cells/μL	30 (69.8%)			
Plasma viral load	5.0 [4.3, 5.5]			
CSF viral load	4.6 [3.6, 5.2]			
Difference in viral loads (Plasma – CSF) log_{10} copies/mL	0.3 [-0.2, 0.9]			
Distribution of Viral Loads log_{10} copies/mL				
	N	Median [IQR]	Mean	P-value
CSF Viral Load	73	4.61 [3.6, 5.15]	4.26	0.03
CSF WBC <5	30	4.74 [3.79, 5.22]	4.54	
CSF WBC >5	13	5.16 [4.71, 5.37]	5.06	
Plasma Viral Load	58	4.99 [4.27, 5.47]	4.61	0.26
CSF WBC <5	25	5.13 [4.46, 5.46]	5.05	
CSF WBC >5	10	5.41 [5.02, 5.63]	5.32	

[1] P-value for mean

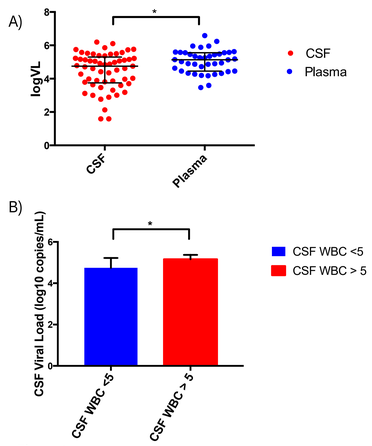


Figure 1.
A) Comparison of viral load in CSF and plasma during cryptococcal meningitis. B) Comparison of the CSF viral load in patients with CSF WBCs < 5 and CSF WBC >5 cells/μL.

Results



Figure 2
A) Phylogenetic tree of a patient with HIV-1 viral load higher in the CSF than plasma. B) Phylogenetic tree of a patient with HIV-1 viral load higher in the plasma compared to CSF. C) Graph demonstrating the p-values of non-parametric tests for population shift in patients with higher viral loads in the CSF versus plasma. D) Graph demonstrating the p-values of parametric bootstrapping test for population migration (Slatkin-Maddison population migration studies) to determine structural shifts between populations in patients with higher viral loads in the CSF versus plasma. E) Graph demonstrating tropism distributions in patients with higher viral loads in the CSF versus the plasma. Patients with population shifts are starred.

A)

	Sample ID	# single genome sequences: CSF	# single genome sequences: Plasma	Population Shift (Yes/No)
Patients with Higher VL in CSF	4040	18	23	Yes
	4063	13	17	No
	4073	30	26	No
	4135	25	16	No
	4229	24	21	Yes
	10120	15	23	No
	10149	30	27	No
	10158	21	12	No
Patients with Higher VL in Plasma	4092	13	25	Yes
	4151	25	15	No
	10046	17	24	No
	10141	13	17	Yes
	10154	20	26	Yes
	10176	20	24	Yes

Parametric panmixia[4,5] – CSF and plasma populations were considered to have a population shift when p-value <0.00005
Non-parametric panmixia[6] – CSF and plasma populations were considered to have a population shift when p-value <0.05

B)

2 x 2 Contingency Table – Population Shift vs VL		
	CSF vs Plasma	
	No Population Shift	Population Shift
Higher VL in CSF	7	2
Higher VL in Plasma	2	4
Fisher Exact; P value = 0.1357		

Table 2.
(A) Single genome sequences derived from CSF and plasma virus. CSF and plasma populations were considered not to be panmictic when the results of parametric and non-parametric tests for population shifts agreed. Analyses for population shift did not include APOBEC hypermutated sequences and identical sequences were collapsed to 1 sequence. (B) 2x2 Contingency table to determine a statistical correlation between population shift and viral loads.

Single genome sequences were obtained of the HIV-1 envelope. In Figure 2C, 2D and Table 2, geographic subdivision and migration studies demonstrate HIV viral compartmentalization between CSF and plasma in 43% (6/14) of patients, 4 of 6 who had higher plasma viral loads and 2 of 8 with higher CSF viral loads.

In Figure 2E, analysis of predicted co-receptors identified X4 variants in CSF and plasma, including patients with compartmentalization; CSF may have predominantly X4 virus even when plasma has predominantly R5 variants.

Conclusion

This is the first description of HIV-1 compartmentalization in the CNS during an AIDS-related infection
- A higher than expected HIV-1 viral load in the CSF of these patients
- Persons with greater CSF pleocytosis had higher CSF viral load
- Of cryptococcal meningitis participants, 43% had distinct HIV populations in the CSF and plasma, with a trend of compartmentalization that varied with CSF viral load
- These data may provide insights into the seeding of the HIV reservoir in CNS opportunistic infections

References

1. Garrido C, Margolis DM. Translational challenges in targeting latent HIV infection and the CNS reservoir problem. *J Neurovirol* 2015. 21: 222-6.
2. Hatano H, et al., Cell-based measures of viral persistence are associated with immune activation and programmed cell death protein 1 (PD-1)-expressing CD4+ T cells. *J Infect Dis*. 2013; 208: 50-6.
3. Gray, LR, et al., Is the central nervous system a reservoir of HIV-1? *Curr Opin HIV AIDS* 2014; 9(6): 552-8.
4. Hudson RR, Boos DD, Kaplan NL. A statistical test for detecting geographic subdivision. *Mol Biol Evol.* 1992; 9: 138-51
5. Achaz G, Palmer S, Kearney M, et al. A robust measure of HIV-1 population turnover within chronically infected individuals. *Mol Biol Evol.* 2004; 21: 1902-12.
6. Slatkin M, Maddison WP. A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics* 1989; *123: 3; 603-613.*

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