CONSERVED CSF HIV ANTIBODY RESPONSE IN PATIENTS WITH DIVERSE NEUROLOGIC PHENOTYPES

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Introduction

The CNS is exposed to HIV during primary infection and likely continuously during untreated chronic infection. ART that suppresses plasma HIV RNA also usually suppresses CSF HIV RNA with occasional asymptomatic episodes of detectable HIV RNA. A rare exception is development of neurosymptomatic (NS) CSF escape in which CNS HIV infection develops despite plasma viral suppression. While drug resistance and incomplete drug penetration predispose to this, the origins of NS escape are not fully understood.

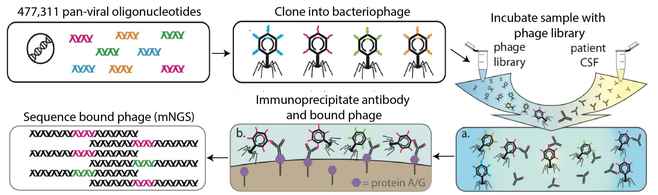
Methods

Patient Demographics

Neurologic status	Treatment status	Number of patients	Number of CSF samples	Additional Information
Variable	Off ART (viremic)	4	16	
Variable	On ART (suppressed)	4	12	
Neurosymptomatic CSF escape	On ART (peripherally suppressed)	4	4	
Secondary CSF escape	On ART (peripherally suppressed)	2	2	varicella zoster virus (n=1), human cytomegalovirus (n=1)
CNS opportunistic infection	Off ART (viremic)	2	8	varicella zoster virus (n=1), HSV-2 n=1
HIV-associated neurocognitive disorder (HAND)	On ART (suppressed)	9	9	

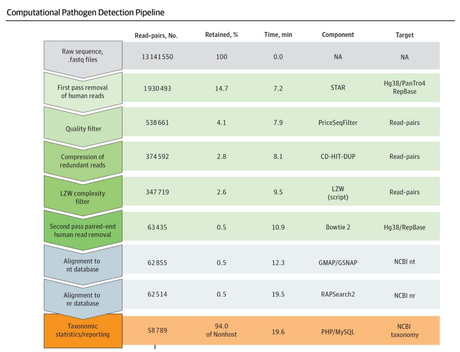
Table 1: CSF samples were obtained from consenting HIV-infected patients with varying treatment and neurologic statuses. Some patients had multiple samples, collected longitudinally, that were included in the study. Additionally, some patients had CNS co-infections, which are noted in the additional information column.

VirScan PhIP Seq



CSF samples were incubated with a VirScan[1,2] T7 bacteriophage library expressing 481,966 peptides tiled across all known vertebrate and arbovirus genomes that previously identified CSF enteroviral antibodies in pediatric acute flaccid myelitis. Antibody-bound phage were immunoprecipitated and after a second round of enrichment, were deep sequenced to quantify enriched viral peptides.

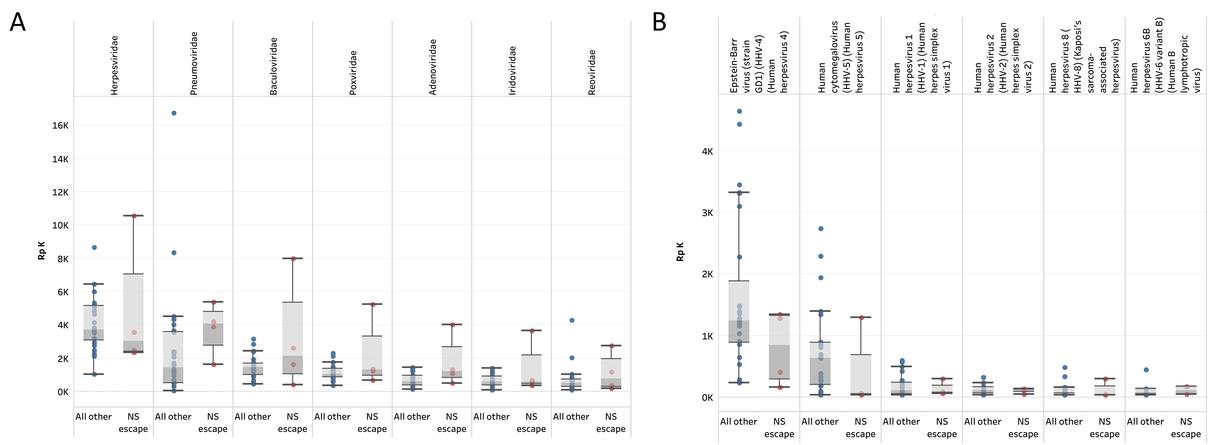
Unbiased mNGS for Pathogen Detection



Separately, total CSF RNA extracted from the cell pellet and unbiased metagenomic next-generation sequencing (mNGS) was performed to look for unidentified infections and HIV. Each sample was sequenced at an average depth of 33.5 million reads. Once sequencing was complete, sequencing reads were put through a custom bioinformatics pathway to filter out human reads, and non-human reads were aligned to their respective genomes and reported as rpM per species on the ID-seq platform.

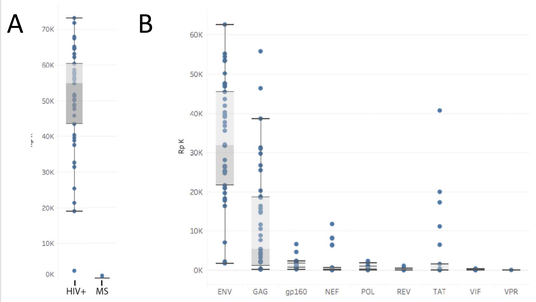
Results

1. No viral reactivation detected by mNGS or pan-viral serology in HIV CSF escape

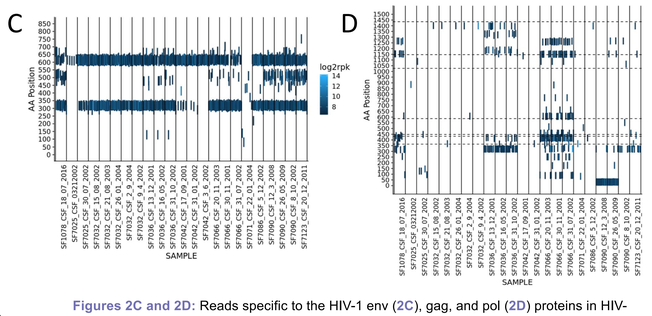


Figures 1A and 1B: CSF samples were incubated with a VirScan[1,2] library (described in methods) and antibodies were immunoprecipitated and antibody-bound phage were sequenced. Reads specific to each viral family were measured were measured and normalized by reads per hundred thousand (rpK) and compared across neurologic phenotypes (1A). Reads specific to each viral species within the family Herpesviridae were measured were measured and normalized by reads per hundred thousand (rpK) and compared across neurologic phenotypes (1B)

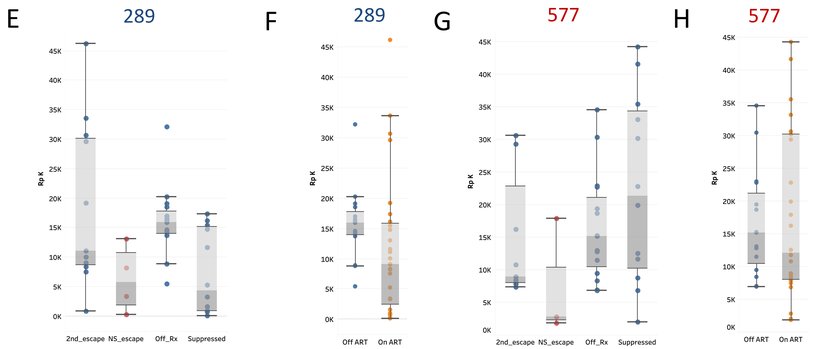
2. Antigenic regions of HIV-1 are highly conserved across treatment groups and neurologic phenotypes



Figures 2A&2B: CSF samples were incubated with a VirScan[1,2] library (described in methods), antibodies were immunoprecipitated, and antibody-bound phage were sequenced. Reads specific to HIV-1 were measured and normalized by reads per hundred thousand (rpK) and compared between HIV-1 infected subjects and HIV-1 non-infected subjects with multiple sclerosis (2A). Reads specific to HIV-1 were further parsed into bins specific for the HIV-1 protein the epitope mapped to (2B)



Figures 2C and 2D: Reads specific to the HIV-1 env (2C), gag, and pol (2D) proteins in HIV-infected CSF samples were further parsed into bins specific for the amino acid position to which they mapped within the reference sequence. Each tick represents a peptide in a single patient that mapped to that specific amino acid position, and the intensity of the color of the line represents the number of log2 reads per hundred thousand sequenced for that particular peptide.



Figures 2E-2H: Reads specific to the HIV-1 env protein in HIV-infected CSF samples were further parsed into bins specific for the amino acid position to which they mapped within the reference sequence, specifically position 289 (2E, 2F) and position 577 (2G, 2H) and compared across treatment groups (2F, 2H) and neurologic phenotypes (2E, 2G)

3. mNGS is sufficient to detect known CNS co-infections

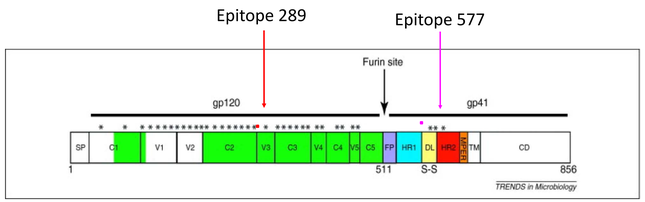
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Patient ID	co-infections	Detected by mNGS?
38069	VZV	yes (3.9 rpM)
5411	CMV encephalitis	yes (104 rpM)
5138	HSV-2 encephalitis	yes (1.1 rpM)
5348	VZV	no

Table 3A: total RNA was extracted from CSF samples from HIV-1 infected subjects with known CNS co-infections, sequenced using unbiased NGS, and run through a custom bioinformatics pipeline to isolate non-human sequences. Numbers of non-human sequences in these samples were quantified and normalized by reads per million (rpM). rpM specific for each known CNS co-infection are recorded in the table above.

Conclusions

- **No evidence was found for reactivation** of another virus in HIV-1 CSF escape in mNGS or pan-viral serologic (VirScan) data
- Positions **289 and 577** on the HIV-1 env protein are common CSF antibody targets and are **conserved** across treatment groups and neurologic phenotypes
- Motif analysis at these positions shows **significant differences/similarities at the sequence level** between neurologic phenotypes and treatment groups



Future Directions

- Fine epitope mapping by mutational scanning of positions 289 and 577
- Comparison of antigenic targeting in matched serum samples to address compartmentalization across neurologic phenotypes
- Examination of additional clinical groups to determine the antigenicity of these regions on the env protein in HIV-1 controller and elite populations

Works Cited

[1] Xu, G. J. et al. Comprehensive serological profiling of human populations using a synthetic human virome. 348, (2015).

[2]Schubert, R. D. et al. Pan-viral serology implicates enteroviruses in acute flaccid myelitis. Nat. Med. 25, (2019).

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