CD4-independent Infection in the CNS of R5 SHIV+ Macaques is Associated with Severe Neuropathology

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Background

We recently reported multifocal giant cell encephalitis (SHIVE) in macaques infected with subtype B and C R5 SHIVs via intravenous, intrarectal or intravaginal inoculation in the absence of immune modulation (**Table 1**). The neuropathology observed in the infected macaques mirrored that of HIVE in infected patients, with several unique and classical HIVE features that are not commonly seen in other SIVE models, including microglial infection[1,2]. The present study aimed to characterize HIV-1 Env variants within the brain of R5 SHIVE macaques with varying degree of neuropathological changes to determine the phenotypes associated with HIV-related CNS infection and injury.

Table 1. Characteristics of subtype B R5 $SHIV_{SF162P3N}$- and subtype C SHIVC109P3- and SHIVC109P3N-infected macaques with SHIVE.

Virus	Animal	Age (yrs)	Mamu allele			Route	Dose ($TCID_{50}$)	Time to AIDS (weeks)	Coreceptor switch (X4 V3 sequence)	SHIV binding antibody
			A*01	B*08	B*17					
$SHIV_{SF162P3N}$	DE86	5.69	-	-	-	IV	3,000	12*	Yes (GHI insertion)	Transient
	T799	9.90	-	-	-	IV	3,000	224	Yes (S11 K/R)	High, persistent
	DG07	6.71	-	-	-	IR	10,000	7*	No	Transient
	DG08	6.98	-	-	-	IR	10,000	20*	Yes(HI/HR insertion, ΔATGD)	Not detected
	DN57	5.84	-	-	-	IR	10,000	30*	No	Weak, persistent
	ET94	6.50	-	-	-	IR	10,000	39	Yes (HR insertion)	Weak, persistent
	DG17	8.85	-	-	-	IVG	1,000	22*	No	Not detected
SHIVC109P3	GC98	7.21	+	-	-	IR	5,000	20*	No	Not detected
SHIVC109P3N	FI08	7.62	-	-	-	IR	5,000	10*	Yes (ΔIGDI)	Not detected

* Indicates rapid progressors. Yrs, years; IV, intravenous; IR, intrarectal; IVG, intravaginal; Δ denotes deletion in the V3 loop.

Methods

Samples from the CSF, blood and brain were collected at the time of necropsy from five encephalitic animals infected with R5 SHIV-B and two encephalitic animals infected with SHIV-C. Full-length envelope glycoprotein gp160 (*env*) was obtained by SGA or bulk PCR. Phylogenetic analyses were performed to genotypically characterize the variants, and single round replication competent viruses were generated and assessed for infection of $CD4^{low}$ and $CD4^{negative}$ cells. Moreover, brain tissue sections were pathologically analyzed using *in situ* hybridization and immunohistochemical techniques.

Results

Lesions in the brain of subtype B R5 SHIVE macaques

Table 2. Histopathologic lesions and staining characteristics in the brain of subtype B R5 $SHIV_{SF162P3N}$-infected macaques with SHIVE.

Animal	Histopathologic Lesions (Type)	Immunohistochemical Staining*		
		SIVp27 (morphology)	CD68/CD163	GFAP
DG07	1	+/-	+/-	ND
T799	1	+ (MΦ)	++	++
DE86	1, 2	++ (MΦ)	++	+
DG08	1, 2	+++ (MΦ)	+++	+
DN57	1, 2	+++ (MΦ)	+++	+
ET94	1, 2, 3	++++ (MΦ; microglia)	+++	++
DG17	1, 2, 3	++++ (MΦ; microglia)	++++	++

*Subjective semi-quantitative scoring (from – to ++++); GFAP, Glial Fibrillary Acidic Protein; MΦ, macrophage; ND, not determined.

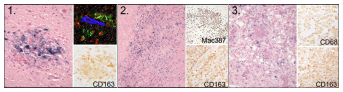


Figure 1. Types of neuropathologic lesions in the brain of subtype B R5 $SHIV_{SF162P3N}$-infected macaques. SIV+ cells as revealed by in situ-hybridization in: **(1)** Cortical perivascular histolytic lesions with multinucleated giant cells (MNGCs), **(2)** thalamic expansive lesions with white matter damage, and **(3)** vacuolated chronic burnt-out lesions. Subsets of CD163+, Mac387+, and CD68+ macrophages are demonstrated by immunohistochemistry (IHC) in insets, with SIV (green), CD68 (red) and glut-1 (blue) shown in confocal inset.

Anatomical and regional *env* compartmentalization in the brain of subtype B R5 SHIVE macaques

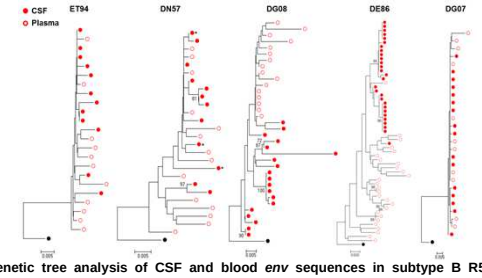


Figure 2. Phylogenetic tree analysis of CSF and blood *env* sequences in subtype B R5 SHIVE macaques. Neighbor-joining phylogenetic trees of *env V3-V5* sequences from the CSF (closed circles) and blood plasma (pen circles) rooted to HIV-1SF162 are shown, with bootstrap values >70% indicated at the appropriate nodes. Genetic distance representing the number of nucleotide substitutions per site between *env* sequences (0.005) is indicated at the bottom of each figure.

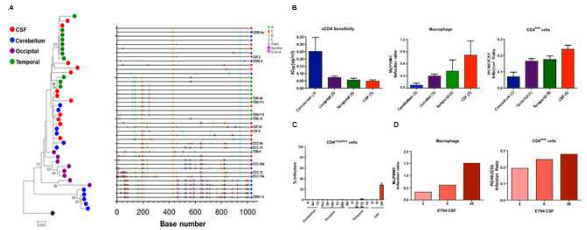


Figure 3. **ET94 Env analysis**. (**A**) Phylogenetic tree and Highlighter Plot analyses of the *env* sequences from the CSF (red), cerebellum (blue), occipital (purple), and temporal (green) regions of ET94. Neighbor-joining tree of *env* V1-V5 region rooted to HIV-1SF162 is shown, with bootstrap values >70% indicated at the appropriate nodes. Genetic distance representing the number of nucleotide substitutions per site between *env* sequences is scaled at the bottom (0.005). Highlighter plot was generated at www.hiv.lanl.gov, and the sequence identifier for the Env clones used in functional studies is indicated on the *y* axis. Positions of nucleotide base transitions and transversions in the highlighter plots are indicated by short, colored coded bars as follows: A: green, T: red, G: yellow. C: blue and gaps: gray. Tics that are bracketed represent G-to-A changes while those in circles represent G-to-A changes in a sequence consistent with an APOBEC signature. (**B**) sCD4 sensitivity, infection of primary macrophages, $CD4^{low}$ and (**C**) $CD4^{negative}$ cells of pseudoviruses expressing Envs from the different brain regions and the CSF compartment. The numbers in parenthesis in panel B indicate the number of clones analyzed per compartment, and the level of infection of CD4 negative cells in panel C is expressed as a percentage of that of cells expressing both CD4 and CCR5. (**D**) Comparison of the ability of the three individual CSF Env clones of ET94 to mediate infection of primary macrophages and $CD4^{low}$ cells. Data are representative of at least two independent experiments.

Emergence of CD4i Env in the brain of subtype B R5 SHIVE macaques

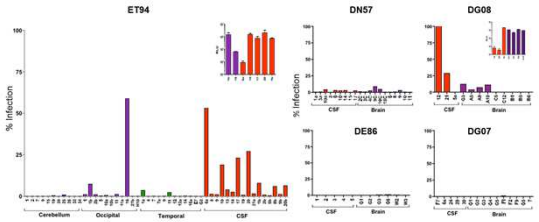


Figure 4. **CD4-independent Envs in the brain and CSF of R5 $SHIV_{SF162P3N}$ SHIVE macaques**. Infection mediated by Envs amplified from the brain and CSF in Cf2Th.CCR5 was assessed, with the level of infection of CD4 negative cells expressed as a percentage of that of $Cf2Th.CD4^{hi}$.CCR5 cells that express both receptors. Insets show absolute relative light units (RLU) in $Cf2Th.CD4^{hi}$.CCR5 cells of select CD4i Env populations from ET94 and DG08.

Env compartmentalization in the brain of subtype C R5 SHIVE macaques

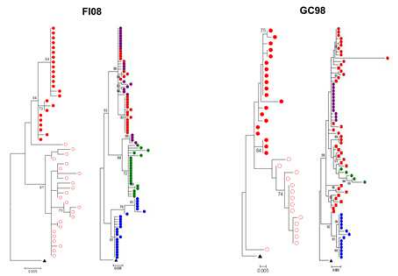


Figure 5. Phylogenetic tree analysis of *env* sequences in the blood and brain of subtype C SHIVE macaques. Neighbor-joining phylogenetic trees of *env V3-V5* sequences from the plasma (open red), CSF (red), cerebellum (blue), occipital (purple) and parietal (green) regions of the brain of SHIVC-infected 'macaques GC98 and FI08 rooted to ZM109F.PB4 are shown, with bootstrap values >70% indicated at the appropriate nodes. Genetic distance representing the number of nucleotide substitutions per site between *env* sequences (0.005) is indicated at the bottom of each figure.

CD4-independent infection in subtype C R5 SHIVE macaques *in vitro* and *in vivo*

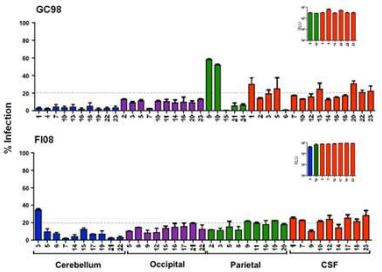


Figure 6. **CD4-independent Envs in the brain of subtype C SHIVE macaques GC98 and FI08**. Env clones amplified from the cerebellum (blue), occipital (purple), parietal (green) region and from the CSF (red) were assessed for their ability to enter Cf2th.CCR5 cells. Infectivity in these CD4-negative cells is expressed as a percentage of that of cells expressing both receptors Data are the mean + s.d. of 2-3 independent experiments. Inserts show infectivity in $Cf2Th.CD4^{hi}$.CCR5 cells of pseudovirions expressing representative high (>20%) CD4i Env populations. RLU, relative light units.

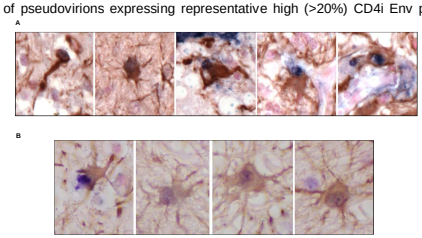


Figure 7. Astrocyte infection in the brain of subtype C SHIVE macaque GC98 (A) and FI08 (B). Immunohistochemistry and in situ hybridization staining of brain sections were carried out (SIV ISH and IHC for GFAP).

Conclusions

- Our data supports an association between severity of neuropathological changes, robust macrophage/microglia infection, evolution to CD4-independence and expanded tropism for truly CD4-negative cells *in vivo*.

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[1] Ren et al., JV87: 6137, 2013

[2] Harbison et al., JNV20: 62, 2014