EARLY EMERGENCE OF MAC-TROPIC HIV VARIANTS IN BRAIN OF PATIENTS WITH NORMAL NEUROLOGY

Poster #365

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Introduction

Highly mac-tropic R5 variants are predominant in brain tissue of AIDS patients with neuroAIDS including HAD. Proviral load in brain tissue is low before neuro-disease develops making it difficult to amplify HIV env sequences. However, if envs could be amplified, they may provide insights into Env genotypes and properties associated with HIV colonization of brain tissue, before the highly mac-tropic, neurovirulent variants develop. Here, we investigated sequences of HIV-1 envs derived from immune and brain tissue of 12 AIDS patients with normal neurology or minor complications (N/MC), before expressing Envs on pseudoviruses and evaluating their properties.

Materials and Methods

Envelopes were amplified from DNA or RNA from single genomes present in brain and spleen, bone marrow or plasma. Envs were cloned into the pcDNA 3.1D/V5-His-TOPO and sequenced. Env+ pseudovirions were prepared by cotransfection of env vectors with env$^-$ pNL4.3 into 293T cells and titrated on HeLa TZM-BL (CD4+ CCR5+ CXCR4+), HIJ (CD4+ CCR5- CXCR4+) and on macrophages. Phylogenetic analyses were undertaken with MEGA v6. Inhibition assays for soluble CD4 (sCD4) were carried out using HeLa TZM-bl cells as target cells.

Results

Table 1. N/MC subjects investigated

Tissues: PBMC: blood; Brain: brain tissue

Patient No.	Collection Year	Brain Bank	Disease status	CD4 count	Viral Load RNA copies/ µl plasma	Viral Load RNA copies/ µl CSF	Therapy
CE161	2004	UCSD	Normal*	11	246,000	2,850	3TC, D4T, KTA, RTV, TFV
CE116	2004	UCSD	MCMD	80	342,386	856,342	D4T, DDI, NFV
CB183	2005	UCSD	Normal	88	74,294	27,400	DDI, TFV
CE148	2004	UCSD	Normal	37	6,026	87,517	3TC, D4T, NFV
CE125	2005	UCSD	Normal	218	-	-	
CE128	2005	UCSD	Normal	10	40,273	587	3TC
CE104	2005	UCSD	Normal	446	33	11	3TC, CBV, IDV
5057	2007	NNABLA	Normal	663	29,282	-	3TC, ABC, ATV, DDI, KTA, RTV, TFV, ZDV
6052	2004	UCLA	Normal	13	75,000	-	3TC, ABC, D4T, EFV, IDV, KTA, NVP
6771	2004	Texas	Normal	23	75,000	2,355	ABC,APV,DDC,EFV,FTV,KTA,T20 ,TFV
8276	2005	Texas	Normal	N/A	750,000	1,101	
10-12	2012	NDRI	PE**		-	-	3TC, AZT

*Normal neurocognitive diagnoses
**Psychotic episodes
TFV, tenofovir; LPV, lopinavir; RTV, ritonavir; KTA, kaletra; 3TC, lamivudine; CBV, AZT/lamivudine; IDV, indinavir; ABC, abacavir; ATV, atazanavir; DDI, didanosine; NVP, nevirapine; APV, amprenavir; DDC, dideoxycytidine; EFV, efavirenz; FTV, fortovase; T20, enfuvirtide; FTC, emtricitabine.

Figure 1. Concentration of tissue DNA required to PCR amplify env genes at endpoint dilutions.

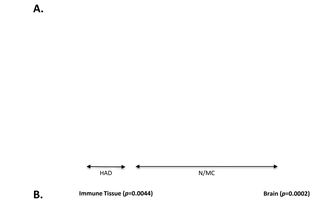


Table 2. Functional Env clones derived from brain and immune tissue

Subject	Env clones from proviral DNA		Env clones for viral RNA	
	Brain	Immune	Brain	Plasma
CE161	10	23	2	10
CE116	12	18	1	NT
10-12	14	16	0	NT
CE104	2	0	0	NT
CE125	2	10	2	NT
CB183	2	15	5	NT
CE128	1	15	1	NT
CE148	0	7	NT	NT
5057	0	14	0	NT
6052	0	8	NT	NT
6771	0	9	0	NT
8276	0	12	1	NT

NT: non-tissue

Figure 3. Representative phylogenetic trees for *env* region in HIV+ subjects. Sequences were aligned with reference sequences representing HIV-1 group M subtype B (http://www.hiv.lanl.gov/)

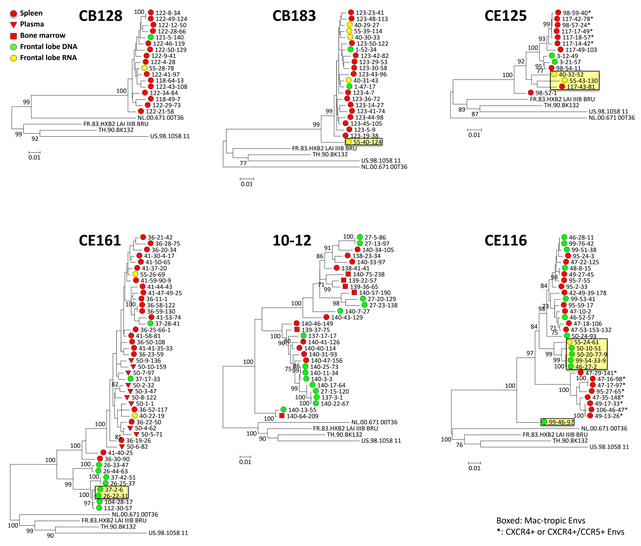


Figure 4. sCD4 inhibition of pseudoviruses expressing mac-tropic and non-mac-tropic R5 Envs from N/MC subjects

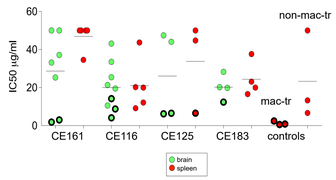


Figure 5. sCD4 inhibition of 10-12 Env+ pseudovirions

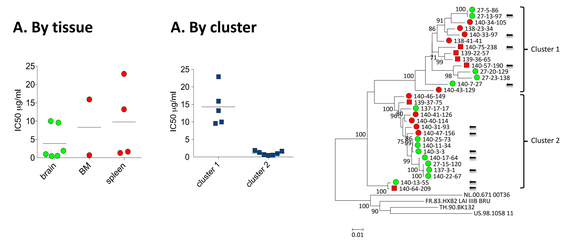
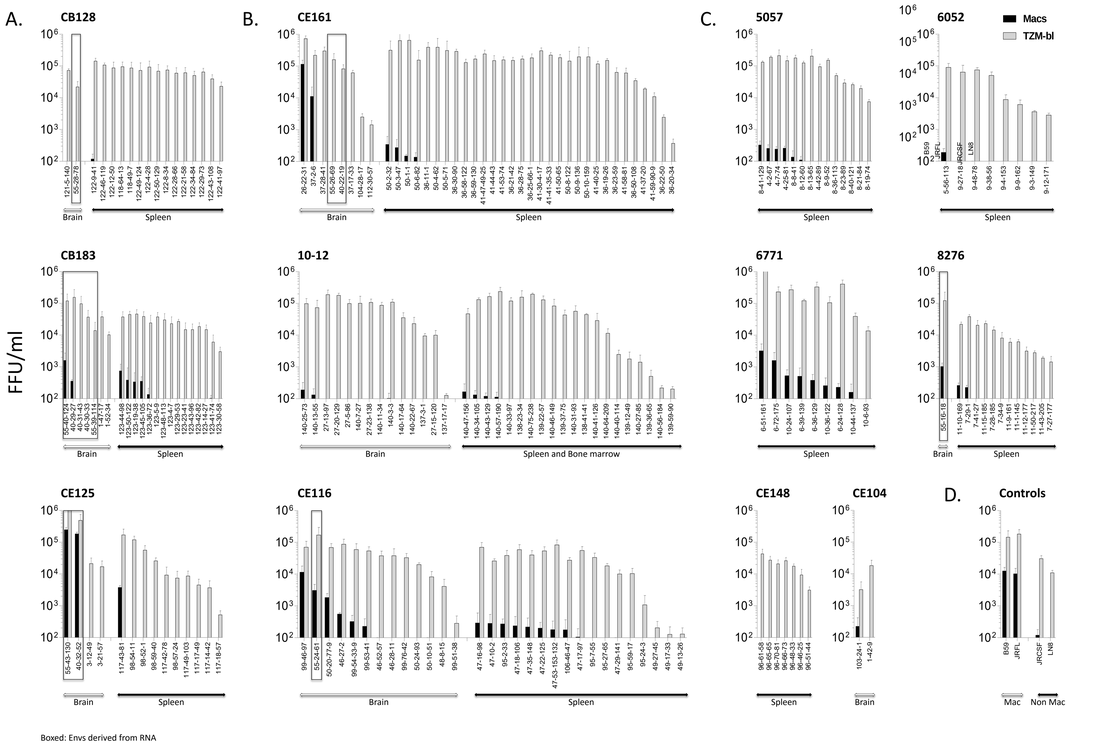


Figure 2. Tropism and receptor-use of envelopes amplified from brain and spleen, blood or bone marrow tissue.



➢N/MC subjects had lower levels of HIV proviral DNA in the brain, with viral sequences difficult to detect in most subjects (figure 1). However, a substantial number of envs were amplified from brain tissue DNA and/or RNA of seven subjects (table 2).

➢Mac-tropic Envs were detected in brain tissue of four N/MC subjects (fig. 2). These mac-tropic R5 Envs in brain tissue formed distinct phylogenetic clusters from most non-mac-tropic envs from spleen or brain (fig. 3). PCR of env sequences from brain tissue RNA indicated that there was evidence of active replication in brain tissue of some subjects.

➢Envs from immune tissue of the N/MC subjects were nearly all tightly non-mac-tropic contrasting with our previous data for neuroAIDS subjects where immune tissue Envs mediated a range of macrophage infectivity from background levels to modest infection, with a small number of Envs mediating high macrophage infection.

➢For subjects CE161, CE116, CE125 and CB183, only the **most** mac-tropic Env+ pseudoviruses were sensitive to sCD4 (Fig. 4). None of the Envs from subject 10-12 were mac-tropic. However, a segregated population of 10-12 Envs from brain, and immune tissue were sensitive to sCD4 (Fig. 5). An enhanced sCD4 sensitivity for these Envs probably indicates an increased Env/CD4 affinity (as it does for mac-tropic Envs).

Conclusions

- ❑ Macrophage-tropic variants are established and likely to be replicating in the brain of a percentage of HIV+ subjects early in disease, well before serious neurological dysfunction becomes apparent.
- ❑ These mac-tropic variants may represent viruses early in their adaptation for infection and replication in brain, perivascular macrophages and microglia.
- ❑ Our data provide new insights into HIV replication and evolution of mac-tropic Envs in brain tissue of AIDS patients before the onset of serious neurological complications.

Acknowledgment

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