Poster # 400

CNS Compartmentalization and Sensitivity to Neutralizing Antibodies

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BACKGROUND

Compartmentalization of HIV-1 has been observed in the cerebrospinal fluid (CSF) of patients with HIV-related neurocognitive disorders (HAND). Compartment specific modifications have been frequently described in the variable loops and the glycosylation sites of the envelope, a known mechanism to escape antibody neutralization. **Considering the low permeability of the blood-brain barrier, we wondered if a lower selective pressure by neutralizing antibodies (NAb) could favor the evolution of NAb-sensitive viruses in the CSF.**

METHODS

- **Single genome amplification (SGA)** was used to sequence near full-length HIV-1 envelope variants (453 sequences) from paired CSF and blood plasma samples of 9 subjects with HAND infected by HIV variants of different clades.
- **Dynamics of viral evolution** were evaluated with a **Bayesian coalescent approach** for individuals with longitudinal samples (n=4).
- For 6 subjects, **infectious pseudotyped viruses** expressing envelope glycoproteins variants representative of the quasispecies present in each compartment were generated, and their **sensitivity to autologous neutralization**, **broadly neutralizing antibodies (bNAbs)** and **sCD4** was assessed.

RESULTS

Subject ID	Age	Sex	Sample date (MM/AA)	Time from/to CSF	Nature	CSF WBC	CD4	VL	number of SGS	Analysis of compartmentalization Fst	Snn	SM	CSF state	Clade
			Jul-11	-288	plasma			5,36	25					
KU	48	M	May-12	0	plasma	0	23	5,77	10	< 0.01	< 0.01	< 0.01	Cp	CRF02_AG
			May-12	0	CSF			4,48	19					
			Jun-13	391	plasma			6,31	28					
			Nov-05	-1978	plasma			4,5	22					
GK	36	F	Apr-11	0	plasma	269	283	5,05	27	< 0.01	< 0.01	< 0.01	Cp	H
			Apr-11	0	CSF			6,52	18					
			Apr-12	367	plasma			5	21					
			Dec-10	-677	plasma			5,99	16					
RO	26	M	Oct-12	-1	plasma	29	48	5,82	19	< 0.01	< 0.01	< 0.01	Cp	C
			Oct-12	0	CSF			5,84	10					
			Oct-11	-1147	plasma			6,06	11					
KP	39	F	Dec-14	0	CSF	28	222	3,66	14	< 0.01	< 0.01	< 0.01	Cp	A1H
			Dec-14	1	plasma			5,79	9					
MG	44	F	Apr-08	-2	plasma	0	-	4,77	21	< 0.01	< 0.01	< 0.01	Cp	B
			Apr-08	0	CSF			4,92	18					
BA	38	F	Mar-14	0	CSF	5	135	4,12	17	0.43	0.21	0.30	Eq	C
			Mar-14	3	plasma			4,52	19					
BL	29	F	Apr-14	0	plasma	8	222	5,75	18	0.21	0.08	0.06	Eq	A1
			Apr-14	0	CSF			5,12	21					
FU	53	M	Nov-13	-2	plasma	0	55	5,87	28	0.87	0.95	1	Eq	B
			Nov-13	0	CSF			4,13	24					
SE	41	F	Apr-09	0	CSF	500	319	5,98	22	0.28	0.29	0.49	Eq	CRF01_AE
			Apr-09	1	plasma			5,11	16					

Table 1: Clinical and virological characteristics of the study subjects and compartmentalization data. Three methods were used to detect genetic compartmentalization between viral populations in the blood plasma and CSF: Slatkin-Maddison test (SM), Wright's measure of population subdivision (F_{st}) and the Nearest-neighbor statistic (S_{nn}): P values <0.01 account for significant genetic compartmentalization; CSF and blood viral populations were found compartmentalized (Cp) based on the phylogenetic trees and if the three compartmentalization tests were concordant (*P* <0.01), or otherwise equilibrated (Eq).

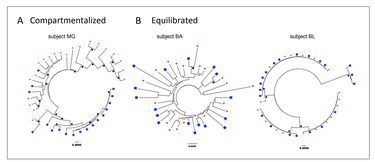

Figure 1. Phylogenetic relationships of paired CSF and blood viral sequences. Neighbor-joining phylogenetic trees representing (A) compartmentalized and (B) equilibrated viral populations. env sequences from the CSF (blue squares) and blood plasma (red triangles) are shown.

1- Study population and SGA results

- Subjects were chronically infected, with a history of past antiretroviral treatments, greatly immuno-suppressed (mean CD4 = 163/mm^3) and hospitalized for neurocognitive conditions by the time of the lumbar puncture.
- Subjects were infected by a wide variety of HIV-1 clades (**Table 1**).

2- Compartmentalization and evolution analysis

- Compartmentalization in the CSF was assessed by examination of phylogenetic reconstruction from paired CSF and blood viral population as well as statistical compartmentalization tests **(Table 1 and Figure 1).**
- 5 of the 9 subjects (55%) showed evidence of CSF compartmentalized viral populations.
- A Bayesian approach was used to study the dynamics of the populations in these chronically infected subjects with neurocognitive disorder **(Figure 2)**.
- CSF compartmentalized populations evolve independently through multiple scenarii including early (2.B) or more recent (2.A, C, D) divergence from their blood counterparts, the latter suggesting recirculation.

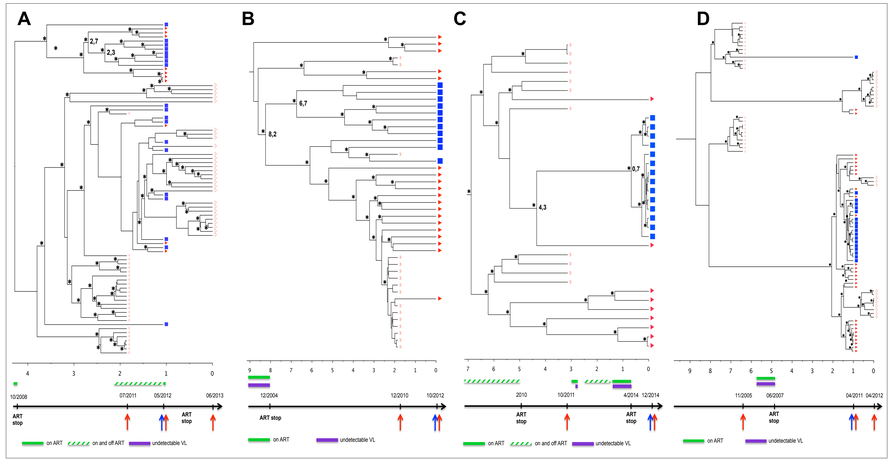

Figure 2. Time scaled Bayesian evolution trees. Subjects KU (A), RO (B), KP (C) and GK (D). Blue squares and red triangles represent CSF and blood plasma derived variants respectively. Asterisks indicate posterior probability > 0.7 and timescale is in years from the last time point e.g. the latest sample.

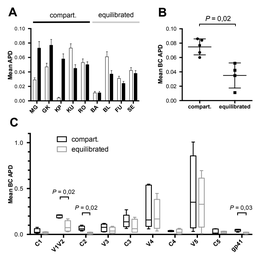

Figure 3. Diversity across envelope glycoproteins.
(A) Mean Average Pairwise Distance (APD) within each compartment. (B) Mean Between Compartments APD (BC APD) across full gp120 and (C) each region.

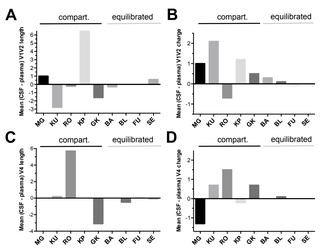

Figure 4. V1V2 and V4 variable regions characteristics.
Length (A, C) and charge (B, D) of V1V2 and V4 respectively.

3- Env molecular characteristics

- Compartmentalized viral populations showed a greater inter-compartment diversity compared to equilibrated populations, especially in V1V2 but also in C2 and gp41 **(Figure 3)**.
- Compartmentalization was associated with extensive modification in length and charge of V1V2 and V4 between CSF and blood plasma sequences **(Figure 4)**.

gp120 domains	C1	V1	V2	C2					V3	C3		V4		C4	V5	C5	gp41					
HxB2	85*		164*	268	270	281	283	290*	295	321	340	363	402	403	440	529		535*	588	630	636	674	684
	V		S	E	V	A	T	N	G	N	Q	T	E	S	E			M	K	E	N	N	L
KU	C								N	D/G		S		A	P			M	K	Q	S	T	I
	P								D/N	G		N		D	Q			I	R	E	N	N	M
GK	C	A	R			K			N		Q		-	-	E	N			K				
	P	N/D	S			G			T/N		E		G/M	T/N	A	D			Q				
RO	C			G				E	K							K/T		V/L					
	P			E				N	V/E							G		L					
KP	C				V	T	T/N		I	D										Q	N	D	M
	P				I	A	N		N	G/D						T				E	Y/S/N	N	I
MG	C			E	I	V	P				N	K	D			N							
	P			G/E	V/I	A/T	N				K	N	N			N							
BA	C																						
	P																						
BL	C												A**					I**					
	P												T					L					
FU	C																						
	P																						
SE	C																						
	P																						

Table 2. Genetic attributes of CSF derived viral sequences. Dominant amino acids (AA) at position with statistical difference between CSF and blood compartments for ≥ 2 subjects or at previously reported position (*) are shown. Bold AA also met Bonferroni adjusted statistical significance. (**) not significant. C : CSF, P : plasma.

- Considering the wide variety of HIV-1 clades in our study population, we looked for CSF specific signature residues in our paired SGA derived viral populations.
- Previously reported key positions were also identified as discriminating, especially in the C2 region (residue 268, 281, 283).
- New key compartmentalized positions were also identified, most notably in C3 and gp41.

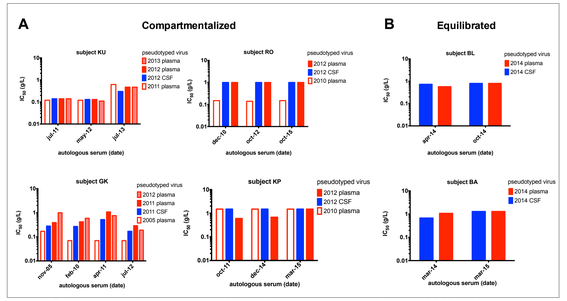

Figure 5. Sensitivity to purified autologous antibodies of pseudotyped viruses from paired CSF and blood plasma samples from subjects with (A) or without (B) CSF compartmentalization. CI_{50} in g/L are represented.

A Compartmentalized	KU			RO			B Equilibrated	BA	
	blood	CSF	ratio	blood	CSF	ratio	blood	CSF	ratio
CD4s	0.526	2.264	**x 4**	7.69	0.22	**x 35**	5.56	13.7	x 2.5
PGT121	0.023	0.201	**x 9**	> 10	8	x 1	1.493	0.015	**x 99.5**
PG16	0.06	2.597	**x 43**	> 10	> 10	-	0.087	0.178	x 2
PGT145	4.267	0.539	**x 8**	0.13	0.023	**x 5.5**	< 0.005	0.075	**x 15**
VRC03	> 10	> 10	-	> 10	> 10	-	0.631	> 10	**x 16**
8ANC195	> 10	> 10	-	> 10	4.167	x 2.5	> 10	> 10	-
10E8	1.37	1.626	x 1	5.128	1.754	x 3	1.449	3.333	x 2.3

	GK			KP			BL		
	blood	CSF	ratio	blood	CSF	ratio	blood	CSF	ratio
CD4s	17.24	11.77	x 1.5	1.31	28.57	**x 22**	6.67	20.41	x 3
PGT121	> 10	> 10	-	> 10	> 10	-	0.546	0.03	**x 18**
PG16	0.04	0.01	**x 4**	> 10	> 10	-	0.496	0.24	x 2
PGT145	0.05	0.018	x 3	> 10	> 10	-	1.449	> 10	**x 7**
VRC03	> 10	> 10	-	> 10	> 10	-	1.163	0.164	**x 7**
8ANC195	> 10	> 10	-	> 10	> 10	-	1.316	1.481	x 1
10E8	3.7	4.17	x 1	0.656	1.111	x 1	> 10	8.696	-

Figure 6. Sensitivity to bNAb and sCD4 of pseudotyped viruses from paired CSF and blood plasma samples from subjects with (A) or without (B) CSF compartmentalization. CI_{50} in µg/mL are represented. CI_{50} ratio is in blue or red indicating a greater resistance of CSF or blood pseudotyped viruses respectively.

4- Sensitivity to autologous neutralization

- We generated infectious pseudotyped viruses from paired CSF and blood plasma samples of 6 subjects and compared their sensitivity to purified IgG from autologous serum at different time points.
- There was no significant difference of sensitivity to autologous neutralization between CSF and blood plasma pseudotyped viruses (**Figure 5**), even for subjects with compartmentalization.

5- Sensitivity to broadly neutralizing antibodies (bNAb) and sCD4

- To further characterize envelope glyco-proteins, we compared their sensitivity to purified bNAb and sCD4.
- A complex profile of neutralization was observed, without any common trend between CSF compartmentalized viruses, nor with regard to the compartmentalization state (**Figure 6**).
- Huge discrepancies were observed between CSF and blood plasma pseudotyped viruses, including subjects with equilibrated viral populations.
- CSF viruses was sometimes more resistant to bNAb, up to 43 times for CSF viruses from subject KU.

CONCLUSIONS

- Our data show that selective pressure by **autologous NAb is not the main driver of HIV evolution in the CSF.**
- Given that each of the conserved neutralizing epitopes is linked to a specific property for cell entry, our data suggest that some functional properties of the envelope are responsible for compartmentalization.
- Considering the possible migration from CSF to blood, **CSF could be a reservoir of bNAb resistant viruses, an observation that should be considered for future studies of immunotherapy.**

ACKNOWLEDGMENTS

This work was supported in parts by the Agence Nationale de Recherche sur le Sida et les hépatites virales (ANRS).

Selected References

Pillai et al. Brain, 2006; Evering et al. Retrovirology, 2014; Sturdevant et al. PloS Pathog, 2015
Chaillon et al. JID, 2014; Arrildt et al. JVI, 2015