HIGH LEVELS OF CELL-ASSOCIATED HIV-1 TRANSCRIPTION IN CSF DESPITE EFFECTIVE cART

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BACKGROUND

- CSF is a possible compartmentalized HIV reservoir, although the cells involved, and their level of HIV-1 transcriptional activity, remain obscure.
- We used a novel highly sensitive assay of HIV-1 RNA/DNA and flow cytometry to study both CSF cells and PBMC, and correlations with MR spectroscopy of brain metabolites.

METHODS

- We studied 10 HIV+ subjects (2 with current HAND) on cART with both plasma and CSF HIV RNA (Roche) <50 copies/ml.
- DNA and RNA were extracted from cells from paired samples of CSF (13-20 ml) and PBMC. Cell-associated HIV-1 transcriptional activity and HIV-1 DNA levels were determined by a newly described quantitative piCode End-Point PCR assay, based on the extremely sensitive piCode MicroDiscs platform (>27-fold sensitivity of real-time PCR; Suzuki et al J AIDS HIV Treat. 2019; 1(2):69). It detects transcripts of HIV LTR, including unspliced RNA (gag/pol), incompletely spliced RNA (tat, vpr, vpu, env), and multiply spliced RNA (rev, nef), with a sensitivity of 3 infected cells/10^6 cells (Fig 1).
- Immunological profiles of CSF cells and PBMC were determined by 18-colour flow cytometry using 5-laser BD Fortessa. CSF and PBMC results were compared by Wilcoxon signed rank test.
- MR spectroscopy evaluated the frontal white matter (FWM), posterior cingulate cortex (PCC), and caudate nucleus.

1. CSF cells had much higher levels of HIV-1 transcriptional activity compared with that in PBMCs
2. Higher levels of CXCR3+ CD49d+ integrinß7- memory CD4 T cells in CSF, compared to PBMC.
3. Inverse-correlation of NAA in FWM and PCC with HIV-1 transcriptional activity in CSF cells.
4. Higher levels of activated CD38+ and/or HLA-DR+ CD4 T cells in CSF, compared to PBMC.

	HIV RNA Copies /10^6 cells	HIV DNA Copies /10^6 cells	Total CSF cell counts Median (IQR)			% of CD4 Median (IQR)		
	Median (IQR)	Median (IQR)	Memory CD4	Memory CD8	Mono-cytes CD14+ CD16+	CXCR3+ CD49d+ Integrin ß7-	CCR5+	CD38+ and/or HLA-DR+
CSF	**4,711** (1647-6780)	**1,314** (427-3804)	**8,818** (2609-21797)	**7,503** (2530-12984)	**455** (206-1453)	**75 %** (71-86)	**48** (40-59)	**18** (7-30)
PBMC	**270** (263-1025)	**752** (236-1025)	N/A	N/A	N/A	**15** (10-19)	**16** (11-22)	**7** (5-11)
p value	*0.004*	0.09	-	-	-	*0.008*	*0.004*	*0.02*

RESULTS

- 9/10 patients' CSF had high levels of cell-associated HIV-1 RNA transcriptional activity (median 4,711 copies/10^6 cells, vs 270 in PBMC; p=0.004; Fig 2, left).
- 8/10 patients had HIV-1 DNA in CSF cells (median 1,314 copies /10^6 cells vs 752 in PBMC; p=0.09).
- 95% of CSF cells were T cells, 95% were memory CD4 and CD8 T cells (median 8,818 and 7,503 cells, respectively). 2.8% were CD14+CD16+ monocytes, 1.7% were NK cells and 0.4 % were B cells.
- CSF CD4 T cells were 75% CXCR3+ CD49d+ integrinß7- (vs 15% of CD4 in PBMC); 48% CCR5+ (vs 16% in PBMC); and 18% expressing CD38 and/or HLA-DR activation markers (vs 7% in PBMC).
- Higher HIV-1 RNA in CSF cells was correlated with lower N-acetyl aspartate in FWM (r=-0.864; p=0.012) and PCC (r=-0.76; p=0.029; Fig 5), respectively.

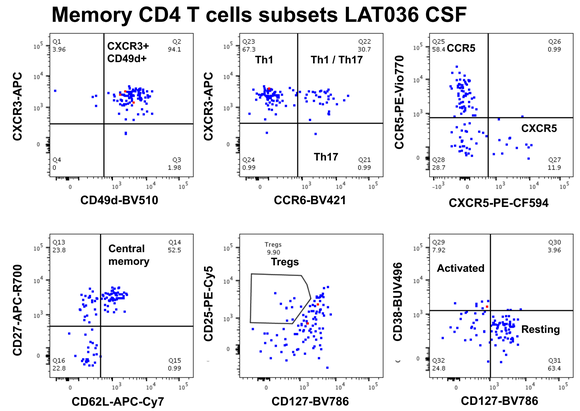


Fig 4. Flow cytometry analysis of CSF memory CD4 T cell subsets showing high expression of CXCR3 and CD49d

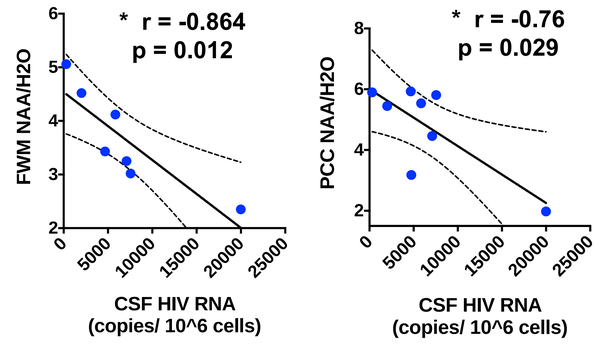


Fig 5. MR spectroscopy shows the inverse correlations between N-acetyl aspartate in FWM, and PCC, and cell-associated HIV RNA, respectively.

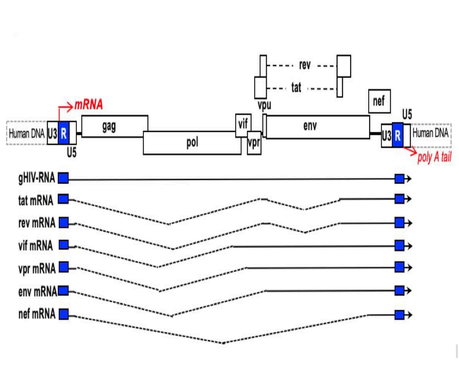


Fig.1 HIV-1 Transcriptional Activity Detection: LTR region targeted to detect all HIV-1 mRNA activity

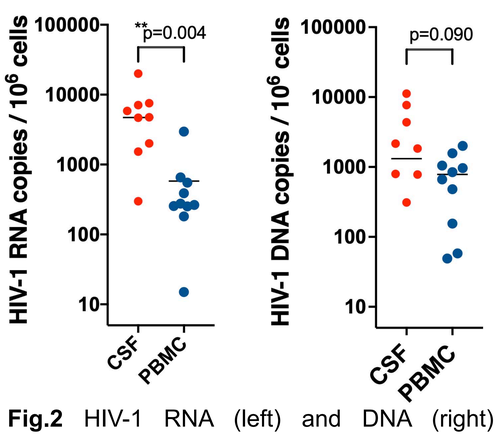


Fig.2 HIV-1 RNA (left) and DNA (right) detection in CSF and PBMC

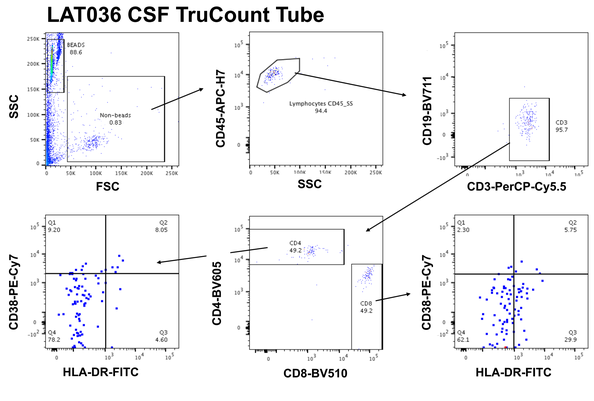


Fig.3 Flow cytometry cell counts of CSF CD4 and CD8 T cells and CD38/HLA-DR activation

CONCLUSIONS

- CSF cellular HIV-1 LTR transcriptional activity is compartmentalised and its biological significance is strongly indicated by the MRS correlations.
- The cellular origin is likely the dominant CXCR3+ CD49d+ integrinß7- non-gut homing memory CD4+T cells; monocytes may be less important.
- Transcriptional products eg tat (vs whole virus) are likely neuropathogenetically significant, given the absence of detectable whole virus and the inverse MRS correlations.
- These data support HIV-1 transcription inhibitor development.

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