

## Cerebrospinal Fluid (CSF) HIV Concentration is Highly Associated with Blood CD8<sup>+</sup> T Cell Activation and Antiretroviral Treatment Responses

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**Background:** The incidence of neurological abnormalities associated with HIV-1 infection has declined substantially since the introduction of combination antiretroviral therapy (ART). However in many patients ART has failed to provide long-lasting plasma viral suppression due to the emergence of drug resistant viral strains. Despite such virological failure ongoing antiviral treatment appears to suppress CSF HIV infection and reduce CSF pleocytosis in many patients. To further characterize CSF cells and their relations to CSF infection and treatment, we developed methods to define CSF lymphocyte phenotypes using 6-color flow cytometry. The % of activated (CD38+HLA-DR+) CD4+ and CD8+ T lymphocytes were measured from paired CSF and blood samples and evaluated in relation to treatment status and CSF HIV.

**General Methods:** This was a cross-sectional analysis at a single time point of subjects participating in a prospective longitudinal study, termed the *Sentinel Neurological Cohort (SNC)*. HIV-infected subjects were assigned to three groups based on treatment and plasma HIV concentrations as outlined in **Table 1**, with an HIV- group serving as controls. Lumbar punctures and other procedures were done in the context of protocols reviewed by the UCSF Committee on Human Research.

**Flow Cytometry Methods:** Six-color flow cytometry was used to measure the levels of activation markers on CD3+CD4+ and CD3+CD8+ T cells. The following antibodies were used to stain paired blood and CSF samples: **CD3 ECD, CD4 PE-CY7, CD8 APC-CY7, HLA-DR FITC, CD38 PE, CD69 APC**. Stained cells were collected on a FACS DIVA and data was compensated and analyzed using FlowJo. Here we focus on concurrent detection of HLA-DR and CD38 expression.

**Virology:** Plasma and CSF HIV RNA levels were measured using the Roche Amplicor PCR assay or its Ultrasensitive modification and a 'floor value' for the lower limit of detection of 19 copies/mL.

**Statistics.** Exploratory analysis of variable correlations used nonparametric (Spearman's) correlations while ANOVA and Student-Newman-Keuls (SNK) post hoc test with alpha = 0.01 were used to compare differences among subject groups. Selected bivariate correlations also used linear regression.

### Results

**Table 1** shows the background characteristics of the subject groups.

Group	Group Definitions		#	Age (years)	Sex (M:F)	Blood T Lymphocytes		CSF WBCs (cells/μL)
	ART	Plasma HIV RNA				CD4+ (cells/μL)	CD8+ (cells/μL)	
HIV Negative (n=1)			14	48 (41-50)	10:4	570 (548-1159)	479 (164-801)	0 (0-2)
HIV Infected	> 3 months	Successes	36	42 (39-49)	34:2	355 (236-554)	884 (706-1147)	2 (0-4)
		Failures	28	43 (37-47)	26:2	228 (214-498)	1042 (656-1373)	2 (0-4)
Off	Native or off > 3 months	no limit	45	41 (36-41)	40:5	352 (232-532)	835 (623-1078)	7 (2-12)

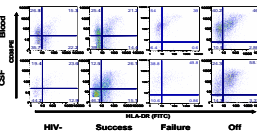
Results presented as median (interquartile range)

**Figure 1** (right) shows examples of cytograms from the four sample groups measuring CD38 and HLA-DR expression in CD8<sup>+</sup> T-cells.

**Figure 2** (below right) shows the HIV RNA concentrations along with the percentage of CD8<sup>+</sup> and CD4<sup>+</sup> activated cells (CD38+DR+).

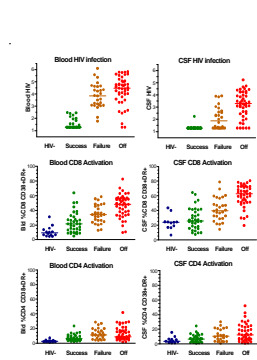
The plasma HIV RNA concentrations of the *Failure* and *Off* groups differed from the *Success* group, but were not significantly different from each other (ANOVA & SNK post hoc test). By contrast the CSF HIV concentrations differed significantly among all three groups (SNK alpha=0.01), indicating a greater effect of "failed" therapy on the CSF than the plasma. The middle panel of **Figure 2** shows that CD8<sup>+</sup> activation was reduced in the *Failures* compared to the *Off* group in both blood and CSF. ANOVA showed this to be significant and post hoc analysis showed CD8<sup>+</sup> activation, in fact, differed in all 4 groups. The same was true in the CSF except that the *Successes* did not differ from the *HIV-* subjects.

As shown in the bottom panel of **Figure 3**, CD4<sup>+</sup> activation was less robust, and there was no difference between the *Off* and *Failure* groups in either blood or CSF.



**Figure 2** (below right) shows the HIV RNA concentrations along with the percentage of CD8<sup>+</sup> and CD4<sup>+</sup> activated cells (CD38+DR+).

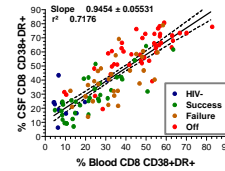
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**Figure 3** (right) shows that across the 3 groups, the % of activated cells in the CSF was highly correlated with those in blood.

Initial exploratory analysis, using simple pair-wise comparisons showed that CD8<sup>+</sup> T-cell activation was highly correlated with CSF HIV concentrations.

Extending this analysis, we used a General Additive Model (GAM, as implemented by Splus) to evaluate the relative contributions of the multiple interacting variables on CSF HIV RNA concentration. This showed a hierarchy of major explanatory variables as follows: **treatment groups > %blood CD8+CD38+HLA-DR+ cells > plasma HIV RNA**. Thus, the activation of CD8<sup>+</sup> T cells is highly associated with the CSF viral load, and appears to be more important than even the plasma viral load.



### Conclusions:

- Multiparameter flow cytometry can be readily applied to CSF lymphocyte subset characterization.
- Antiretroviral therapy has a beneficial effect on CSF HIV infection,
  - Not only in those with plasma viral suppression (in whom CSF suppression is similar)
  - But also in those for whom treatment fails to completely suppressing plasma HIV
- Failed treatment also reduces T cell activation in both blood and CSF. The schematic to the right outlines one hypothesis relating these two findings.
- This hypothesis suggests that partial suppression of HIV and emergence of less-fit, pathogenetically attenuated HIV might lead to less robust immune activation and less transfer of systemic infection to the CNS.
- CD8<sup>+</sup> T cell activation in CSF parallels that in blood.

Finally, in order to examine whether differences in lymphocyte activation might explain the greater effect on CSF than plasma HIV in the *Failures* group, we examined the relationship between CD8<sup>+</sup> T-cell activation and viral loads in these two patient groups.

The analysis in **Figure 4** (right) suggests a difference in this relationship in the two fluids:

1. If systemic infection determines lymphocyte activation, this activation is less robust in the *Failures* (perhaps due to decreased fitness or pathogenicity of resistant viruses) than in those *Off* treatment.
2. In CSF the level of infection is determined by that of peripheral immune activation, i.e., the causal relationship is reversed.

