

CSF Pleocytosis Is Associated with Viral Trafficking from Blood into CNS

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Background

Previous studies comparing HIV isolates from plasma and cerebrospinal fluid (CSF) have demonstrated variable compartmentalization. However, few studies have characterized longitudinal viral dynamics between blood and CSF. Pleocytosis, a marker of inflammation, is common in HIV infection. We hypothesized that during pleocytosis, trafficking of HIV-infected lymphocytes into CSF would result in genetic equilibration of sequences derived from blood and CSF.

Objective

- To determine changes in compartmentalization of blood and CSF viral isolates associated with pleocytosis.
- To determine rates of co-receptor usage of CSF and blood viral isolates.

Methods

Chronically infected HIV-1 patients, who interrupted their anti-retroviral treatment, were intensively investigated. Viral load changes in paired blood and CSF samples were documented along with the development of CSF pleocytosis. Viral sequences encoding the C2V3 loop of *env* were amplified by nested RT-PCR from paired blood and CSF samples. Replicate PCR products were cloned and sequenced. Phylogenetic analysis was performed using fDNAML on clonal sequences (PhyIip 3.5 and G. Olsen). Assessment of degree of inter-compartment segregation was performed by calculating posterior probability values using linear discriminant analysis and multidimensional scaling. Co-receptor usage was determined using support vector machine (SVM) method (<http://genomic2.ucsd.edu:8080/wetcat/v3.html>).

Figure 1. Subject 686 with no pleocytosis throughout maintained viral compartmentalization

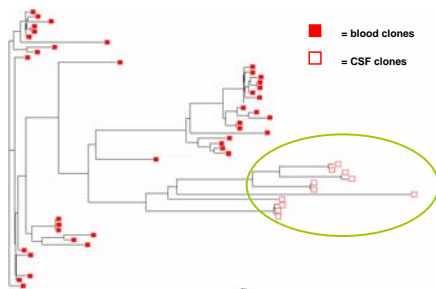


Figure 1A. *env* sequences derived from blood and CSF of subject 686 remained phylogenetically distinct when sampled longitudinally over 3 weeks, $p < 0.01$ per Slatkin Madison test.

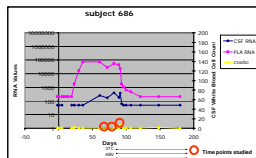


Figure 1B (left). Time course of viral loads in blood and CSF compartments and white blood cell (WBC) counts in CSF. Figure 1C (below). Multidimensional scaling analysis of genetic distances of viral sequences. M equals median of posterior probabilities of compartmentalization, i.e., the closer to 1.00, the higher degree of segregation.

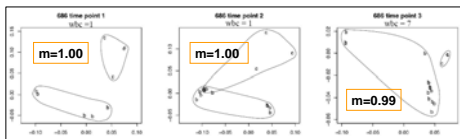


Figure 2. Subject 839 demonstrated that developing pleocytosis disrupted viral compartmentalization

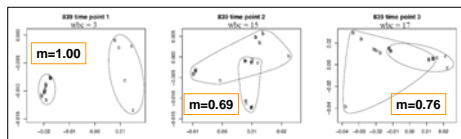


Figure 3. Subject 487 demonstrated that persistent pleocytosis revealed no compartmentalization

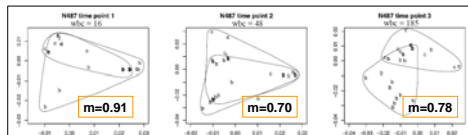


Table 1. Demographic characteristics of study subjects and the coreceptor usage of their viruses

Subject No.	Age	Gender	Race	CD4 count (cells/ml)	Peak Pleocytosis (cells/ml)	CCR5 co-receptor usage(%)
686	43	Male	African American	200	7	61
839	58	Male	African American	293	17	99
487	52	Male	White	461	185	100

Results

- CSF pleocytosis is associated with disruption of viral compartmentalization.
- Viruses with identical sequences were isolated from both blood and CSF concurrent with pleocytosis (data not shown), suggesting viral trafficking between the blood and CSF compartments.
- Most viruses isolated from both compartments used CCR5 as co-receptor, and there was no significant difference in co-receptor usage between compartments.

Summary and Conclusions

- Inflammation, marked by CSF pleocytosis, allows virus immigration from blood to CSF.
- This viral trafficking may increase the overall viral genetic diversity within the CSF.
- Further investigation will be needed to determine how inflammation, pleocytosis and viral trafficking influence neuropathogenesis associated with HIV infection.

Acknowledgments

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