CNS Inflammation During Treatment-Induced HIV Suppression

Abstract #1607
Poster 491

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Introduction

- Central nervous system (CNS) inflammation and immune activation are characteristic features of untreated HIV-1 infection that is reflected by alterations in the concentration of inflammatory mediators in cerebrospinal fluid (CSF). With antiretroviral treatment (ART) the elevated concentrations of these inflammatory biomarkers are characteristically reduced.
- While ART most often reduces both plasma and CSF HIV RNA levels to below the limit of detection by clinical assays, more sensitive assays have detected evidence of low level of HIV RNA in both plasma and CSF in 'well-treated' patients.
- We have reported that low levels of CSF HIV RNA detected by the single copy assay (SCA) were associated with mild elevations of CSF neopterin, a pteridine metabolite associated with immune activation, particularly with macrophage activation.
- In order to further characterize CNS immune activation in treated patients, we measured a panel of 9 additional inflammatory biomarkers in the CSF in the group of treatment-suppressed subjects in which low-level virus had also been measured by single copy assay (SCA) in both CSF and paired plasma samples.

Methods

Study Design. Archived samples of CSF and plasma from 45 HIV-positive subjects with >2 years of treatment and viral suppression (<40 HIV RNA cpm) by standard clinical assay were included. Only subjects on 2011 DHHS preferred antiretroviral regimens were evaluated. Of the 45 subjects, 18 had repeated CSF and plasma sampling so that a total of 76 HIV positive sample pairs were included. For comparison purposes, 21 age matched HIV uninfected controls were included. Samples were included from two centers in San Francisco, CA and Gothenburg, Sweden.

Measurements. Both CSF and blood viral load was further evaluated using the sensitive SCA method to measure virus levels down to <0.3 cpm. Biomarkers of inflammation and neuropathogenesis were measured in cerebrospinal fluid (CSF) by luminex cytokine and chemokine array and ELISA. Five different assays were used including Milliplex high sensitivity cytokine multiplex (TNF-a and IL-6; Millipore, Billerica, MA), Milliplex standard sensitivity cytokine multiplex (IP-10 and MCP-1), Milliplex cardiovascular disease panel 1 (MMP-9 and sVCAM), Milliplex Timp Panel 2 (TIMP-1), sCD163 ELISA (R&D Systems) and sCD14 (R&D Systems). Results were acquired by Luminex 200, analyzed by Bioplex 6.1 and compiled using Bioplex Datapro (Bio-Rad). CSF neopterin was measured by ELISA (BRAHMS Aktiengesellschaft, Henningsdorf, Germany). Additionally, CSF neurofilament light chain protein (NFL) was measured using a sensitive sandwich ELISA method (NF-light® ELISA kit, UmanDiagnostics AB, Umeå, Sweden) to assess neural injury.

Analysis. CSF biomarker results in the treated-suppressed samples were compared to age-matched HIV-uninfected controls. Additionally, the CSF samples with <0.3 RNA cpm (SCA-) were compared to those with >0.3 cpm (SCA+) to see if detection of low level CSF virus associated with enhanced intrathecal inflammation. All comparisons used unpaired Mann-Whitney's test. All graphs show box-plots with 10-90 percentile whiskers and mean values indicated by "+".

Results

Figure 1. Comparison of CSF biomarker concentrations between subjects with undetectable plasma and CSF HIV RNA by conventional assay (<40 copies/mL) and an HIV-uninfected control group. Of the CSF biomarkers assessed, significant elevations in the treated-suppressed group compared to HIV- controls were detected only in CSF TNF-α and CSF sCD163. There were no significant differences between treated-suppressed and HIV- subjects in other inflammatory biomarkers or NFL.

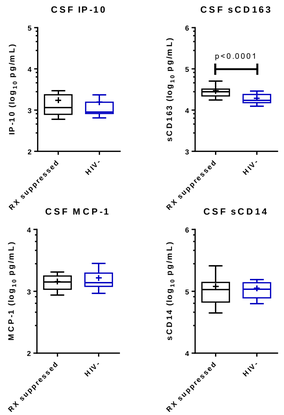


Table 1. Baseline Subject Characteristics
Medians (interquartile ranges), or absolute number in each group are shown

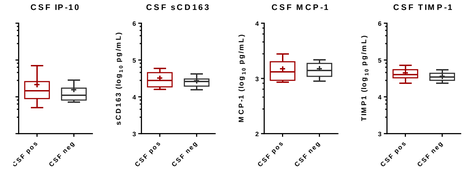
	RX suppressed	HIV negative
Subjects (N)	45	21
Site (SF:GOT)	30:15	21:0
Gender (M:F)	40:5	15:6
Age (years)	48 (43-56)	47 (44-50)
Time of Infection (years)	8 (4-13)	-
Time of suppressive therapy (years)	3 (1-5)	-
Nadir CD4	193.5 (53-280)	-
Pre-ART Plasma RNA (log_{10}cpm)	4.8 (4.3-5.5)	-
Pre-ART CSF RNA (log_{10}cpm)	3.7 (2.6-4.2)	-
CD4 (cells/μL)	613 (390-730)	878 (690-1116)
CD8 (cells/μL)	803 (618-1087)	446 (329-746)
CSF WBC (per μL)	2 (1-3)	1 (0-3)
CSF:blood Albumin ratio	5.24 (4-7)	5.5 (3.8-7.6)

Approximate normal values in CSF: WBC < 5 cell/ul; CSF:blood Albumin ratio 4.6 (+/-1.3) for age 41-50, 4.0 +/-1.1 for age 31-40

HIV RNA Analysis by Single Copy Assay (SCA)

- In 45 subjects with undetectable viral load by standard clinical assay, HIV-1 RNA was detected by SCA in 6% of the subject's CSF and in 20% of the subject's plasma at baseline.
- Upon analysis of all 76 samples, including 18 subjects with repeated measures, the extraction control failed for 6 CSF and 8 plasma measures, leaving 70 CSF and 68 plasma from 45 subjects for analysis.
- Twelve of the 70 (17%) CSF samples were positive for HIV-1 RNA which was significantly less than the 39/68 (57%) positive plasma samples (p<0.0001).

Figure 2. Comparison of CSF biomarkers between subjects with SCA-detected CSF HIV RNA and those below detection (<0.3 copies/mL) within the HIV+ group shown in Figure 1. Unlike our previous study of CSF neopterin, no difference was found between the SCA+ and SCA- subgroups for any of the CSF biomarkers.



Summary & Conclusions

- **In 45 subjects with viral suppression (<40 cpm) by preferred regimens, there were significant elevations in the treated-suppressed group compared to HIV uninfected controls in CSF TNFα and sCD163 but not in the other biomarkers studied.**
  - This suggests that some subjects exhibit persistent, though restricted low-level, inflammation in the presence of preferred ART regimens.
  - These results differ from previous studies examining this issue.
- **When we compared all CSF samples with positive HIV RNA (SCA+, >0.3cpm) against CSF samples with negative HIV RNA (SCA-, <0.3 cpm), there was little evidence of ongoing viral replication in relation to CSF immune activation.**
  - Low levels of CSF HIV RNA by SCA did not correlate with elevation of biomarkers of CNS inflammation.
- **The reasons for the difference between these results and those of other studies may relate to:**
  - choice of biomarkers
  - selection of subjects, in this case subjects with low levels of residual HIV RNA in both plasma and CSF

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