Title

Biomarkers of chemotaxis and inflammation in cerebrospinal fluid and serum in individuals with HIV-1 subtype C versus B

Journal

Journal of NeuroVirology, 22(6)

ISSN

1355-0284

Authors

de Almeida, Sergio M
Rotta, Indianara
Jiang, Yanxin
et al.

Publication Date

2016-12-01

DOI

10.1007/s13365-016-0437-4

Peer reviewed

HHS Public Access

Author manuscript

J Neurovirol. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

J Neurovirol. 2016 December ; 22(6): 715–724. doi:10.1007/s13365-016-0437-4.

Biomarkers of Chemotaxis and Inflammation in Cerebrospinal Fluid and Serum in Individuals with HIV-1 Subtype C versus B

Sergio M. De Almeida[1,2], **Indianara Rotta**[1,2], **Yanxin Jiang**[3], **Xiao Li**[4], **Sonia M. Raboni**[1], **Clea E. Ribeiro**[1], **Davey Smith**[5], **Michael Potter**[8], **Florin Vaida**[6], **Scott Letendre**[5], **Ronald J. Ellis**[7,8], and **the HNRC Group**[8]

[1]Universidade Federal do Paraná, Curitiba, Paraná, Brazil

[2]Faculdades Pequeno Príncipe, Curitiba, Paraná, Brazil; Instituto de Pesquisa Pelé Pequeno Príncipe, Curitiba, Paraná, Brazil

[3]Alzheimer's Therapeutic Research Institute, University of Southern California, San Diego, CA, USA

[4]Chicago Cleaning House, Chicago, IL, USA

[5]Division of Infectious Diseases, Department of Medicine, University of California, San Diego, CA, USA

[6]Division of Biostatistics and Bioinformatics, Department of Family Medicine and Public Health, University of California, San Diego, CA, USA

[7]Department of Neurosciences, University of California, San Diego, CA, USA

[8]HIV Neurobehavioral Research Center, University of California, San Diego, San Diego, CA, USA

Abstract

A defective chemokine motif in the HIV-1 Tat protein has been hypothesized to alter central nervous system cellular trafficking and inflammation, rendering HIV-1 subtype C less neuropathogenic than B. To evaluate this hypothesis, we compared biomarkers of cellular chemotaxis and inflammation in cerebrospinal fluid (CSF) and serum in individuals infected with HIV-1 subtypes B (n=27) and C (n=25) from Curitiba, Brazil. None had opportunistic infections.

Corresponding Author: Sérgio Monteiro de Almeida, MD, PhD, Hospital de Clínicas – UFPR, Seção de Virologia, Setor Análises Clínicas, Rua Padre Camargo, 280, Curitiba – PR – Brasil, 80060-240, sergio.ma@ufpr.br, Telephone/Fax: +55 (41) 3360-7974.

Part of this work was previously presented at the 13th International Symposium on NeuroVirology and 2015 Conference on HIV in the Nervous System, June 2–6, 2015, San Diego, California, USA.

Conflict of Interest:
SERGIO M. DE ALMEIDA , the author declare that have no conflict of interest.
YANXIN JIANG , the author declare that have no conflict of interest.
INDIANARA ROTTA, the author declare that have no conflict of interest.
XIAO LI , the author declare that have no conflict of interest.
SONIA M. RABONI , the author declare that have no conflict of interest.
CLEA E. RIBEIRO , the author declare that have no conflict of interest.
DAVEY SMITH , the author declare that have no conflict of interest.
MICHAEL POTTER , the author declare that have no conflict of interest.
FLORIN VAIDA , the author declare that have no conflict of interest.
SCOTT LETENDRE , the author declare that have no conflict of interest.
RONALD J. ELLIS , the author declare that have no conflict of interest.

Chemokines (MCP-1, MIP-1α, MIP-1β, RANTES, IP-10) and cytokines (TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-10) were measured using the multiplex bead suspension array immunoassays or ELISA HD. CSF and serum biomarker concentrations were compared between subtype B and C groups and HIV-positive and HIV-negative subjects (N=19) using an independent group *t*-test (unadjusted analysis) and linear regression (adjusted analysis), controlling for nadir CD4 and CSF and plasma HIV RNA suppression. CSF levels of cytokines and chemokines were significantly ($p < 0.05$) elevated in HIV-positive versus HIV-negative participants for 7/13 biomarkers measured, but levels did not differ for subtypes B and C. Serum levels were significantly elevated for 4/13 markers, with no significant differences between subtypes B and C. Although pleocytosis was much more frequent in HIV-positive than in HIV-negative individuals (27% vs. 0%), subtypes B and C did not differ (32% and 22%; $p = 0.23$). We did not find molecular evidence to support the hypothesis that intrathecal chemotaxis and inflammation is less in HIV-1 subtype C than in subtype B. Biomarker changes in CSF were more robust than in serum, suggesting compartmentalization of the immunological response to HIV.

Keywords

biomarkers; inflammatory; HIV associated neurocognitive disorders (HAND); HIV-1; subtype; cerebrospinal fluid (CSF)

INTRODUCTION

HIV type 1 (HIV-1) is characterized by extensive genetic diversity and has evolved into major subtypes that differ in prevalence by geographical region. Regional variation is based, in part, on founder effects. For example, India and sub-Saharan Africa host predominantly subtype C, whereas in Europe and the U.S., subtype B dominates. Subtypes are characterized by structural and functional differences that can influence cellular tropism and organ involvement. If such differences affect central nervous system (CNS) disease, this could result in geographic variation in the prevalence of cognitive impairment (Kanki et al. 1999; Kaleebu et al. 2002). A clinical study reported that dementia was rare in India, where subtype C predominates (Satishchandra et al. 2000), although other groups, applying standardized neuropsychological tests, found high rates of cognitive impairment among HIV-1subtype C participants in South India (Gupta et al. 2007) and Brazil (de Almeida et al., 2013; Gupta et al., 2007).

The Trans-Activator of Transcription (Tat) protein in HIV-1 subtype B viruses bears a CC dimotif that confers strong chemotaxis for monocytes and increases the production of immune mediators (cytokines and chemokines) *in vitro* (Weiss et al. 1999; Woodman et al. 1999; Park et al. 2001). In contrast, HIV-1 subtype C Tat shows no such chemotaxis and cytokine stimulation, due to the replacement of one cysteine residue in the CC dimotif with a serine (CS, SC) (Ranga et al. 2004). This alteration could influence neurovirulence by reducing cellular trafficking and neuroinflammation in subtype C infections compared to B infections. Previous studies have evaluated cellular chemotaxis and neuroinflammatory biomarkers only in subtype B (Genis et al. 1992; Brabers and Nottet 2006; Cinque et al. 2007; Yuan et al. 2013; Peluso et al. 2013).

The purpose of the present study was to compare indicators of cellular trafficking and inflammation in the CSF and serum of individuals with HIV-1 subtypes B and C infections, and HIV negative individuals. All patients were recruited from a single region of southern Brazil where both B and C subtypes are common.

Biomarkers were selected for this study on the basis of their roles in leukocyte migration from the peripheral circulation into the CNS and their direct or indirect stimulation by HIV tat (Nath 1999; King et al. 2006; Campbell et al. 2007a; Abbas and Herbein 2013). Chemokines are a group of small molecular weight (8–14 kDa) proteins (Zlotnik and Yoshie 2000); the CC- or β-chemokines are characterized by the presence of two adjacent cysteines near their amino terminus (Laing and Secombes 2004). Specifically, we studied four β-chemokines stimulated by the C30C31 motif of HIV-1 Tat: MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (CCL5). In addition we measured the α-chemokine C-X-C motif chemokine, type 10 (CXCL10), also known as interferon gamma-induced protein type 10 (IP-10). The cytokines TNF-α, IFN-y, IL-1β, IL-2, IL-4, IL-6, IL-7 and IL-10 were measured due to their role in neuroinflammation and in the CNS HIV pathobiology (Genis et al. 1992; Nath 1999). To the authors' knowledge, there are no studies addressing the difference of HIV-1 B and C cytokines and chemotactic stimulation.

RESEARCH DESIGN AND METHODS

Subjects

Fifty two HIV-positive research volunteers were recruited at the Hospital de Clínicas, Universidade Federal do Paraná (UFPR), Curitiba, Paraná, Brazil. None had opportunistic infections in the CNS. All volunteers provided samples of blood and cerebrospinal fluid (CSF) under a study protocol approved by the Hospital de Clínicas-UFPR (Brazil) Institutional Review Board (IRB) and the Brazilian National IRB (CONEP). All HIV-positive participants underwent serological testing to confirm their HIV status before enrollment according to guidelines published by the Brazilian Ministry of Health (Brasil 2009). As lumbar punctures could not be performed in HIV-negative subjects in Brazil, we recruited a comparison group of 19 HIV-negative individuals at the HIV Neurobehavioral Research Center (HNRC) under a protocol approved by the IRB at the University of California, San Diego (UCSD). The HIV-negative group was matched by age to the HIV-positive group. All participants were free of neurological co-morbidities and had negative serological tests for hepatitis C virus and syphilis. As described previously (de Almeida et al., 2013).

Methods

Lumbar punctures were performed using an atraumatic spinal needle and aseptic technique. CSF total protein was determined by benzethonium chloride and glucose by hexoquinase/G-6-PDH (both Architect, Abbott, IL). Total leukocytes/mm^3 was determined in fresh, uncentrifuged CSF by manual counting in a Fuchs-Rosenthal chamber. CSF pleocytosis was defined as leukocytes > 5 cells/mm^3. For differential leukocyte counts, CSF samples were concentrated by Shandon Cytospin (Pittsburgh, USA). The slides were then stained by May

Grünwald-Giemsa technique. Aliquots of CSF and serum were frozen and stored at −80°C for later batch testing of cytokine and interleukin levels.

Clinical laboratory measures

HIV RNA levels in serum and CSF were measured by branched DNA assay (Siemens) with a nominal limit of detection of 50 copies/mL. CD4 counts were quantified by flow cytometry (FACSCalibur-Multitest). Nadir CD4 levels was extracted from medical records; as described previously (de Almeida et al., 2013). Albumin levels in CSF and serum were determined by the nephelometry (Dade Behring BNII, Deerfield, IL). The CSF/serum albumin quotient, QAlb = Alb CSF/Alb serum, was used to assess blood-CSF barrier function. The upper limit of the reference range of QAlb is age-dependent; it was calculated for each case by the following: QAlb ref = (4 + age (y)/15) × 10^{-3} (Reiber and Peter, 2001).

HIV subtyping

For participants who had clinical resistance, the HIV genotyping was assigned using *pol* sequences. For the remainder, the subtype was determined by sequencing *env* from HIV DNA. Together these yielded 27 subtype B and 25 subtype C infections (de Almeida et al., 2013; Rotta et al., 2014).

CSF and serum biomarker quantification

Biomarkers in CSF and serum were quantified by multiplex bead suspension array immunoassays (EMD Millipore, Billerica, MA), which used antibodies added to dyed fluorescent microspheres (TNF-α, IFN-y, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-10, MCP-1, MIP-1α, MIP-1β, IP-10) or high sensitivity enzyme linked immunosorbent assay (RANTES; R&D Systems, Minneapolis, MN). The sensitivity of these assays ranged between 0.1 to 10.1 pg/mL. All samples were assayed concurrently in duplicate according to the manufacturers' instructions.

Statistical analyses

Demographic data, HIV disease characteristics, and CSF biochemical, cytological, and virological measures were compared between individuals with subtype B and C infections using the independent samples *t*-test for continuous variables and Fisher's exact test for binary and categorical variables (gender, AIDS diagnostic, ART, HCV serostatus, HIV RNA in plasma and CSF). The demographic data and CSF biochemical and cytological measures were compared between the HIV-positive (subtypes B and C combined) and the HIV-negative control groups using similar methods. The distribution of plasma HIV viral loads was highly skewed so the Wilcoxon rank-sum test was used.

The CSF and serum biomarker values were $\log_{10}$-transformed to normalize their distributions, and presented in terms of mean (SD), since they are approximately normal on the log scale. The biomarkers were then compared between subtype B and C groups. We used a multivariable linear regression (adjusted analysis), controlling for plasma HIV viral load suppression, which was statistically different between the subtypes, and nadir CD4 count. Because low nadir CD4 has been shown in previous studies to be associated with increased soluble biomarkers of inflammation and chemotaxis in HIV (Gisslen et al., 1994;

Mooney et al, 2015; Noel et al., 2014), we included nadir CD4 in the multivariable model (Vittinghoff, 2011). Results were considered statistically significant at the 5% alpha level. Cohen's d effect sizes (and 95% confidence intervals) were reported for differences between groups. These were calculated as the ratio of the mean difference between groups to the residual standard deviation of the linear model.

RESULTS

As shown in Table 1, subtype B- and C-infected individuals were similar in age, gender, and education. Subtype B-infected participants had lower nadir CD4 counts (83 versus [vs.] 175) and a longer estimated duration of HIV infection than the subtype C-infected. Subtype B-infected participants were somewhat more likely to be on antiretroviral therapy (ART) (89% vs. 64%; p = 0.05). Participants taking ART were more likely to be virologically suppressed than those not taking ART (plasma HIV RNA < 50 copies/mL, 81% vs. 40%, p = 0.01). ART included non-nucleoside reverse transcriptase inhibitors (NNRTIs) in 9/21 (43%) of those with subtype B infections vs. 4/15 (27%) of those with subtype C infections (p = 0.48). Also shown in Table 1 are HIV disease characteristics and hepatitis C virus (HCV) serostatus according to subtype.

1. CSF profile and HIV RNA levels

Table 2 shows CSF biochemical, cytological and virological characteristics for HIV-negative and HIV-positive subtype B and C individuals. CSF pleocytosis (increased leukocytes of >5 cells/mm^3) was significantly more frequent in HIV-positive (27%) than HIV-negative (0%) subjects (p= 0.015). Subtypes B- and C-infected subjects did not differ significantly either in the frequency of pleocytosis (6 (22%) vs. 8 (32%); p = 0.43) or in absolute numbers of CSF leukocytes (median, IQR: 1.6 (0.3, 4.9) vs. 3.4 (0.6, 11); p = 0.23).

Median CSF HIV RNA levels were higher (and the proportion detectable was lower) in subtype C than B infections, although this difference did not reach significance (p = 0.06). As noted previously, subtype C participants were somewhat less likely to be on ART. Plasma HIV RNA was significantly higher in subtype C- than in B-infected individuals, and there were fewer cases with undetectable plasma HIV RNA in subtype C (Table 2).

In CSF, total protein, albumin, and albumin quotient (QAlb) were all significantly higher in HIV-positive participants than HIV-negative controls. Subtypes B and C did not differ on these measures (Table 2). CSF glucose was significantly higher in HIV-positive than HIV-negative group; and in HIV-1 subtype B than C, but in all groups CSF glucose levels were under reference range.

2. Inflammatory biomarkers

Comparison between HIV-positive and HIV-negative groups—CSF levels of cytokines and chemokines were significantly elevated (p< 0.05) in HIV-positive versus HIV-negative participants for 7 of the 13 markers measured. By contrast, serum levels were significantly elevated in only 4 of 13 markers. Differences between HIV positive and HIV negative participants, assessed as effect sizes, were larger on average for CSF than for serum (Table 3).

CSF levels of the cytokines TNFα, IFNα, IL-7, and IL-10 were significantly elevated in HIV-positive patients compared with those of HIV-negative individuals, whereas, in serum TNFα and IFNα levels were significantly elevated in HIV-positive individuals. CSF levels of the chemokines IP-10, MCP-1, and MIP-1α were significantly elevated in HIV- positive compared with HIV-negative individuals, whereas, in the serum of HIV- positive patients, only IP-10 and RANTES were significantly elevated (Table 3).

Comparison of HIV-1 subtypes B and C—For both unadjusted (data not shown) and adjusted (Table 4) analyses, cytokine and chemokine levels in CSF and serum did not differ significantly for subtypes B and C. Additionally, CSF/serum ratios were not significantly different. As described previously, adjusted analyses were performed for specific covariates (viral suppression on ART [plasma HIV RNA < 50 copies/mL] and CD4 nadir). Table 4 shows mean biomarker values adjusted for these covariates, plus effect sizes, measured as the standardized difference (Cohen's d) between subtypes B and C. No significant differences were seen in any of the biomarkers in serum or CSF. In CSF, effect sizes were small (d < 0.22) for all markers except IL-2 (d = 0.45; p=0.15). In serum, effect sizes were small to medium (0.02 – 0.53), and none approached statistical significance, save IFNα (d = 0.53; p=0.09).

CSF/serum ratio: The CSF/serum IFNα and RANTES ratios were higher in HIV-positive than in HIV-negative individuals (p = 0.001 and 0.004, respectively). For all of the other biomarkers, there were no significant differences (all p-values >0.05; data not shown).

DISCUSSION

CNS immunological response in individuals with HIV-1 subtype B and C infections

We detected many significant differences in the levels of cytokines or chemokines in CSF and serum between HIV positive and negative individuals, but no differences were found between subtypes B and C. Although lower chemoattractant activity was reported previously in HIV-1 subtype C infections than in subtype B infections, this was not found in the present study. CSF WBC count was slightly higher in HIV-1 subtype C-infected individuals than those with subtype B, although not statistically significant. In conjunction with our finding that the β-chemokines studied have similar values in CSF and serum of HIV-1 subtype B- and subtype C-infected individuals, we conclude that, *in vivo*, there was no difference in the stimulation of β-chemokines, including MCP-1, in CSF and serum of HIV-1 subtype B- and subtype C-infected individuals. The levels of IP-10, an α-chemokine, were also similar in both the HIV subtype B and HIV subtype C groups.

As we discuss in detail below all these results must be carefully interpreted due to the differences in treatment and HIV suppression between the participants groups. In the subtype C group, there were fewer cases with undetectable CSF VL; plasma HIV RNA levels were higher; and there were fewer cases with undetectable plasma HIV RNA levels than subtype B group. This could be explained by the fact that in the subtype C group there were fewer patients on ART and fewer patients with AIDS than in subtype B.

Our findings contrasts with the *in vitro* observation that subtype C Tat protein fails to induce MCP-1 production. Numerous laboratory studies have shown reduced activation of proinflammatory cytokines (Mishra et al. 2008a; Mishra et al. 2008b; Gandhi et al. 2009), decreased augmentation of TNF-α (Campbell et al. 2007a), and less severe cognitive impairment in mice with the C31S substitution (Rao et al. 2008; Rao et al.2013). These studies provided evidence of reduced neuropathogenesis in subtype C, secondary to the C31S substitution. This was not confirmed by the present *in vivo* analysis.

The literature on biomarkers in HIV-1 non-B subtype infections is scarce, and in the available studies, these biomarkers are not compared among different subtypes. To date, this is the first study to attempt to define HIV inflammatory and cellular biomarker differences in subtype B and C infections *in vivo*, as well as study these markers in different compartments, CSF and serum.

Early clinical reports of cognitive outcomes related to HIV-1 subtype C infections identified very low rates of dementia among subtype C-infected individuals in India versus individuals infected with subtype B in North America (Teja et al. 2005). The lower frequency of HIV-associated dementia (HAD) in India was interpreted as evidence that HIV-1 subtype C is less neurovirulent than subtype B (Riedel et al. 2006). A study performed in Alberta, Canada, found the prevalence of HAND in HIV-1 subtype B twice the prevalence in subtype C; although the majority of HIV-1 subtype C participants were immigrants with different cultural backgrounds and origins and different native languages (Pornpun et al. 2012). However, other groups found high rates of cognitive impairment among HIV subtype C-infected individuals (de Almeida et al., 2013; Gupta et al., 2007).

The influx of WBC to CSF is not related to a single protein; rather, a complex interaction of host proteins, cytokines and chemokines (Sherry et al. 1998), as well as HIV proteins (King et al. 2006) are involved in this function. To explain the absence of a difference in CSF WBC in HIV-1 subtype B and C infections, we hypothesized that *in vivo* Tat deletion could not lead to the loss of its chemoattractant property stimulating MCP-1 properly. This hypothesis and the fact that in a previous study (de Almeida et al. 2013) we did not find differences in neuropsychological impairment between subtype-B and C infected individuals led us to investigate MCP-1 and other inflammatory biomarkers in CSF and serum.

A previous study also found no difference in CSF HIV RNA levels between subtype B and C infections. This same study also investigated differences in CSF markers (HIV-1 RNA, and neopterin) in HIV-1 subtypes (A, B, and C) and recombinant forms (AE and AG), but did not find any subtype-dependent differences. The authors found a significant subtype-dependent difference in the CSF WBC count between the subtype B and C groups, higher in subtype C infections, but this result was not verified in a multivariate analysis. However, in contrast to our study, this study did not analyze inflammatory and chemotaxis biomarkers. (Abdulle et al. 2008).

Monocyte chemotaxis driven by Tat is mediated by both direct and indirect processes. Several *in vitro* studies demonstrated direct chemotaxis using recombinant Tat protein, and this activity of Tat has been related to the C30C31 dicysteine motif in Tat (Albini et al. 1998;

Beall et al.1996). Such a dicysteine motif is also a key feature of β-chemokines (Allavena et al.1994; Albini et al.1998). An *in vitro* comparison of HIV-1B and HIV-1C Tat proteins showed that B Tat induces robust monocyte migration and induces β-chemokines, while HIV-1 C Tat is only a weak inducer (Rao et al. 2008). The absence of a dicysteine motif in HIV-1 C Tat is considered to be responsible for the reduced chemotactic function (Ranga et al.2004). Using chemically synthesized Tat proteins, Campbell et al. (2007a) showed that HIV-1 B Tat protein binds to CCR2, induces an intracellular calcium flux, and causes monocyte chemotaxis, as well as induces MCP-1 and TNFα. In contrast, Tat from a Southeast Asian HIV-1 subtype C, without a dicysteine motif, did not. The same group showed that Tat protein from HIV-1C isolate retaining a dicysteine motif was competent to induce monocyte chemotaxis and induced MCP-1 and TNFα to levels similar to that observed with HIV-1B Tat protein (Campbell et al. 2007b). In our study CSF TNF-α and IL-10 were higher in the HIV-1 subtype C group than in the HIV-1 subtype B group, although the difference did not reach statistical significance. TNF-α induces the production of MCP-1and IP-10 (Sheng et al. 2005).

Our data suggest that, *in vivo*, there is no decrease in chemotactic function in HIV-1 subtype C, despite our expectation based on previous *in vitro* and animal studies. The fact that CSF WBC counts did not differ between subtype B and C infections, paired with the previous clinical finding that there is no difference in neurocognitive impairment between the two subtypes (de Almeida et al. 2013), strongly suggest that HIV-1 subtype C is no less neurovirulent than subtype B. A careful analysis of the CSF samples in this study provides important information on HIV subtype diversity, CNS impairment, and compartmentalization.

Comparison of the immunological responses in physiological compartments, CSF, and blood in HIV infections

In the HIV-positive and HIV-negative groups, the immunological response differed between the CSF and serum compartments. Our data indicates that more cytokines and chemokines were stimulated in the CSF than in the serum.

Thus, we conclude that the immunological response is more intense in the CSF than it is in the serum. The differences we found in the quantification of inflammatory biomarkers were dependent on the presence of HIV infection; these differences were not seen in the HIV-negative group.

CSF showed a more intense response than serum, with a mix of pro and anti-inflammatory (IL-10) markers, although a predominance of pro-inflammatory markers (TNF-α, IFN-y). IL-7 was also higher in CSF. This cytokine is an important inducer of autoimmune (Th-17) reactions. In serum, there was exclusively pro-inflammatory (TNF-α, IFN-y) cytokines. This supports the view that the CNS is a main site of HIV infection and corroborates the hypothesis that although the CNS is considered an immunologically privileged site (Barker et al. 1977; Kreutzberg 1996), due to specific immunological and constitutional characteristics of the CNS, it reacts in an adequate way when stimulated. The CNS was viewed as a site protected by the blood-brain barrier (BBB), now it is known to be a dynamic immunological environment through which immune cells migrate to prevent and respond to

events such as infection (Banks 2014). Rather than the concept of the BBB being a purely anatomical barrier, it is now considered a highly reactive interface controlled by signals from capillary endothelial cells, glial cells, pericytes, and neurons in the CNS, as well as from immune responses in the periphery (Abbott 2010; Zhang et al. 2009; Huber et al. 2014). The less important immunological response in serum than CSF could be related to the fact that the majority of the participants of this study are on HAART and are HIV suppressed; these will reinforce more the concept of the CNS as isolated immunological compartment. Waida et al (2015) measuring biomarkers of inflammation and immune activation in serum but not in CSF, observed that most biomarkers were relatively normalized in the suppressed group compared with the naïve group. Although, residual immune activation, particularly monocyte/macrophage activation persisted.

This study's findings should be interpreted with caution due to the possibility of confounding and type II error. We did not find significant differences between subtypes B and C in biomarkers of inflammation and intrathecal chemotaxis. With respect to confounding, the subtypes differed in their distribution of HIV disease and treatment characteristics. Thus, study subjects with subtype C infections were, on average, less likely to be virologically suppressed on ART than subtype B. In addition, the subtype C group had less severe historical levels of immunosuppression as measured by nadir CD4. Because low nadir CD4 has been shown in previous studies to be associated with increased soluble biomarkers of inflammation and chemotaxis in HIV (Gisslen et al., 1994; Mooney et al., 2015; Noel et al., 2014), we included nadir CD4 in a multivariate model (Vittinghoff, 2012). The statistically adjusted the effect size estimates presented in Table 4 show no subtype differences.

In the case of MCP-1 (CCL2), the chemokine hypothesized to be directly influenced by the defective Tat motif and the one believed particularly important in cellular trafficking into the CNS, the estimated subtype effect sizes were very small and did not approach significance. Additionally, across the panel of cytokines and chemokines measured, the direction of the subtype differences (columns 4 and 8 of Table 4) was in many cases opposite to that which would be predicted by a deficit in chemotaxis resulting from the hypothesized defective subtype C Tat motif (i.e., higher levels for subtype B vs C). In contrast to the subtypes, we did detect numerous significant differences of medium effect size between HIV+ and HIV- subjects (Table 3). In all cases, these differences were as expected: higher biomarker levels in HIV+ than in HIV−. The combined sample size for the subtypes was somewhat smaller (N=52) than for HIV serostatus (N=71). Therefore smaller effect sizes for the subtypes could not be ruled out.

Another possibility is that subtype C Tat in Brazil does not bear the same defective chemokine motif as subtype C in the previous *in vitro* experiments from India. In India, the C30C31S substitution is present in more than 90% of HIV-1 subtype C cases (Ranga et al. 2004). We do not know the frequency of subtype C Tat C30C31S substitution in Brazil or in the samples studied, and future studies are needed to determine these frequencies.

Some biomarkers previously shown to be abnormal in HIV, such as neopterin and neurofilament light (NFL), were not measured in this study. We focused on markers of

intrathecal chemotaxis and inflammation, rather than macrophage activation or neuronal injury.

Conclusions

Chemokine and interleukin levels were frequently elevated in the CSF of HIV-positive subjects compared with those of HIV-negative patients; the differences were dependent on the presence of HIV infection. We did not find any molecular evidence to support the hypothesis that intrathecal chemotaxis in HIV-1 subtype C infections is less than in subtype B infections. Pleocytosis, a marker of cellular trafficking, was equally common in subtype B- and C-infected individuals. The immunological response in CSF and serum in HIV infection were qualitatively different in the two compartments.

Acknowledgments

This work was supported by the following grants: National Institute of Health, NIH R21 MH76651 (Ellis, Ronald J; Almeida, Sergio M.), NIH R01 MH83552 (Smith, David M.), S10 RR31646 (Letendre, Scott), K24 MH097673(Letendre, Scott); University of California, San Diego, Center for AIDS Research (CFAR), an NIH-funded program (P30 AI036214), which is supported by the following NIH Institutes and Centers: NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NIA, NIGMS, and NIDDK; Ministério *da* Ciência e Tecnologia/Conselho Nacional de Desenvolvimento Científico e Tecnológico, MCT/CNPq-Universal 014/2008, Brazil (Almeida, Sergio M.).

The HIV Neurobehavioral Research Center (HNRC) is supported by Center award P30MH062512 from NIMH.The San Diego HIV Neurobehavioral Research Center [HNRC] group is affiliated with the University of California, San Diego, the Naval Hospital, San Diego, and the Veterans Affairs San Diego Healthcare System, and includes: Director: Robert K. Heaton, Ph.D., Co-Director: Igor Grant, M.D.; Associate Directors: J. Hampton Atkinson, M.D., Ronald J. Ellis, M.D., Ph.D., and Scott Letendre, M.D.; Center Manager: Thomas D. Marcotte, Ph.D.; Jennifer Marquie-Beck, M.P.H.; Melanie Sherman; Neuromedical Component: Ronald J. Ellis, M.D., Ph.D. (P.I.), Scott Letendre, M.D., J. Allen McCutchan, M.D., Brookie Best, Pharm.D., Rachel Schrier, Ph.D., Debra Rosario, M.P.H.; Neurobehavioral Component: Robert K. Heaton, Ph.D. (P.I.), J. Hampton Atkinson, M.D., Steven Paul Woods, Psy.D., Thomas D. Marcotte, Ph.D., Mariana Cherner, Ph.D., David J. Moore, Ph.D., Matthew Dawson; Neuroimaging Component: Christine Fennema-Notestine, Ph.D. (P.I.), Monte S. Buchsbaum, M.D., John Hesselink, M.D., Sarah L. Archibald, M.A., Gregory Brown, Ph.D., Richard Buxton, Ph.D., Anders Dale, Ph.D., Thomas Liu, Ph.D.; Neurobiology Component: Eliezer Masliah, M.D. (P.I.), Cristian Achim, M.D., Ph.D.; Neurovirology Component: David M. Smith, M.D. (P.I.), Douglas Richman, M.D.; International Component: J. Allen McCutchan, M.D., (P.I.), Mariana Cherner, Ph.D.; Developmental Component: Cristian Achim, M.D., Ph.D.; (P.I.), Stuart Lipton, M.D., Ph.D.; Participant Accrual and Retention Unit: J. Hampton Atkinson, M.D. (P.I.), Jennifer Marquie-Beck, M.P.H.; Data Management and Information Systems Unit: Anthony C. Gamst, Ph.D. (P.I.), Clint Cushman; Statistics Unit: Ian Abramson, Ph.D. (P.I.), Florin Vaida, Ph.D. (Co-PI), Reena Deutsch, Ph.D., Anya Umlauf, M.S.

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, nor the United States Government.

REFERENCES

Abbas W, Herbein G. T-cell signaling in HIV-1 Infection. Open Virol J. 2013; 7:57–71. [PubMed: 23986795]

Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. Neurobiol Dis. 2010; 37:13–25. [PubMed: 19664713]

Abdulle S, Hagberg L, Svennerholm B, Fuchs D, Gisslen M. Cerebrospinal fluid viral load and intrathecal immune activation in individuals infected with different HIV-1 genetic subtypes. PloS One. 2008; 3:1–5.

Albini A, Benelli R, Giunciuglio D, Cai T, Mariani G, Ferrini S, Noonan DM. Identification of a novel domain of HIV tat involved in monocyte chemotaxis. J Biol Chem. 1998; 273:15895–15900. [PubMed: 9632634]

Allavena P, Bianchi G, Zhou D, van Damme J, Jilek P, Sozzani S, Mantovani A. Induction of natural killer cell migration by monocyte chemotactic protein-1, −2 and −3. Eur J Immunol. 1994; 24:3233–3236. [PubMed: 7805752]

Banks WA. The blood-brain barrier in neuroimmunology: tales of separation and assimilation. Brain Behav Immun. 2014; 44:1–8. [PubMed: 25172555]

Barker CF, Billingham RE. Immunologically privileged sites. Adv Immunol. 1977; 25:1–54. [PubMed: 345773]

Beall CJ, Mahajan S, Kuhn DE, Kolattukudy PE. Site-directed mutagenesis of monocyte chemoattractant protein-1 identifies two regions of the polypeptide essential for biological activity. Biochem J. 1996; 313(Pt 2):633–640. [PubMed: 8573103]

Brabers NACH, Nottet HSLM. Role of the proinflammatory cytokines TNF-alpha and IL-beta in HIV-associated dementia. Eur J Clin Investig. 2006; 36:447–458. [PubMed: 16796601]

Brasil. AIDS e Hepatites Virais. DST Dd. Ministério da Saúde: Programa Nacional de DST/AIDS; 2009. http://www.aids.gov.br/assistencia/manualdst/item12.htm

Campbell GR, Watkins JD, Singh KK, Loret EP, Spector SA. Human immunodeficiency virus type 1 subtype C Tat fails to induce intracellular calcium flux and induces reduced tumor necrosis factor production from monocytes. J Virology. 2007a; 81:5919–5928. [PubMed: 17376903]

Campbell EM, Perez O, Melar M, Hope TJ. Labeling HIV-1 virions with two fluorescent proteins allows identification of virions that have productively entered the target cell. Virology. 2007b; 360:286–293. [PubMed: 17123568]

Cinque P, Brew BJ, Gisslen M, Hagberg L, Price RW. Cerebrospinal fluid markers in central nervous system HIV infection and AIDS dementia complex. Handb Clin Neurol. 2007; 85:261–300. [PubMed: 18808988]

de Almeida SM, Ribeiro CE, de Pereira AP, Badiee J, Cherner M, Smith D, Maich I, Raboni SM, Rotta I, Barbosa FJ, Heaton RK, Umlauf A, Ellis RJ. Neurocognitive impairment in HIV-1 clade C- versus B-infected individuals in Southern Brazil. J Neurovirol. 2013; 19:550–556. [PubMed: 24277437]

Gandhi N, Saiyed Z, Thangavel S, Rodriguez J, Rao KVK, Nair MP. Differential effects of HIV type 1 clade B and clade C Tat protein on expression of proinflammatory and anti-inflammatory cytokines by primary monocytes. AIDS Res Hum Retroviruses. 2009; 25:691–699. [PubMed: 19621989]

Genis P, Jett M, Bernton EW, Boyle T, Gelbard HA, Dzenko K, Keane RW, Resnick L, Mizrachi Y, Volsky DJ, Epstein LG, Gendelman HE. Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV disease. J Exp Med. 1992; 176:1703–1718. [PubMed: 1460427]

Gisslen M, Chiodi F, Fuchs D, Norkrans G, Svennerholm B, Wachter H, Hagberg L. Markers of immune stimulation in the cerebrospinal fluid during HIV infection: a longitudinal study. Scand J Infect Dis. 1994; 26(5):523–533. [PubMed: 7855550]

Gupta JD, Satishchandra P, Gopukumar K, Wilkie F, Waldrop-Valverde D, Ellis R, Ownby R, Subbakrishna DK, Desai A, Kamat A, Ravi V, Rao BS, Satish KS, Kumar M. Neuropsychological deficits in human immunodeficiency virus type 1 clade-C-seropositive adults from South India. J Neuro Virol. 2007; 13:195–202.

Huber AK, Duncker PC, Irani DN. Immune responses to non-tumor antigens in the central nervous system. Front Oncol. 2014; 13:328.

Johnson TP, Patela K, Johnson KR, Marica D, Calabresi PA, Hasbun R, Nath A. Induction of IL-17 and nonclassical T-cell activation by HIV-Tat protein. Proc Natl Acad Sci U S A. 2013; 110:13588–13593. [PubMed: 23898208]

Kaleebu P, French N, Mahe C, Yirrell D, Waters C, Lyagoba F, Nakiyingi J, Rutebemberwa A, Morgan D, Weber J, Gilks C, Whitworth J. Effects of human immunodeficiency virus (HIV) type 1 envelope subtypes A and D on disease progression in a large cohort of HIV-1-positive persons in Uganda. J Infect Dis. 2002; 185:1244–1250. [PubMed: 12001041]

Kanki PJ, Hamel DJ, Sankale JL, Hsieh C, Thior I, Barin F, Woodcock SA, Gueye-Ndiaye A, Zhang E, Montano M, Siby T, Marlink R, Ndoye I, Essex ME, Mboup S. Human immunodeficiency virus type 1 subtypes differ in disease progression. J Infect Dis. 1999; 179:68–73. [PubMed: 9841824]

King JE, Eugenin EA, Buckner CM, Berman JW. HIV Tat and neurotoxicity. Microb Infect. 2006; 8:1347–1357.

Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996; 19:312–318. [PubMed: 8843599]

Laing K, Secombes CJ. Chemokines. Dev Comp Immunol. 2004; 28:443–460. [PubMed: 15062643]

Mishra M, Vetrivel S, Siddappa NB, Ranga U, Seth P. Clade-specific differences in neurotoxicity of human immunodeficiency virus-1 B and C Tat of human neurons: significance of dicysteine C30C31 motif. Ann Neurol. 2008a; 62:366–376.

Mishra A, Gordon VD, Yang L, Coridan R, Wong GC. HIV TAT forms pores in membranes by inducing saddle-splay curvature: potential role of bidentate hydrogen bonding. Angew Chem Int Ed Engl. 2008b; 47:2986–2989. [PubMed: 18338358]

Mooney S, Tracy R, Osler T, Grace C. Elevated Biomarkers of Inflammation andCoagulation in Patients with HIV Are Associated with Higher Framingham and VACS Risk Index Scores. PLoS One. 2015; 10(12):e0144312. [PubMed: 26641655]

Nath A. Pathobiology of human immunodeficiency virus dementia. Semin Neurol. 1999; 19(2):113–27. [PubMed: 10718533]

Noel N, Boufassa F, Lecuroux C, Saez-Cirion A, Bourgeois C, Dunyach-Remy C, Goujard C, Rouzioux C, Meyer L, Pancino G, Venet A, Lambotte O. ANRS C021 CODEX Study Group. Elevated IP10 levels are associated with immune activation and low CD4(+) T-cellcounts in HIV controller patients. Aids. 2014; 28(4):467–476. [PubMed: 24378753]

Park IW, Wang JF, Groopman JE. HIV-1 Tat promotes monocyte chemoattractant protein-1 secretion followed by transmigration of monocytes. Blood. 2001; 97:352–358. [PubMed: 11154208]

Paul RH, Joska JA, Woods C, Seedat S, Engelbrecht S, Hoare J, Heaps J, Valcour V, Ances B, Baker LM, Salminen LE, Stein DJ. Impact of the HIV Tat C30C31S dicysteine substitution on neuropsychological function in patients with clade C disease. Journal of Neurovirology. 2014; 20:627–635. [PubMed: 25366660]

Peluso MJ, Meyerhoff DJ, Price RW, Peterson J, Lee E, Young AC, Walter R, Fuchs D, Brew BJ, Cinque P, Robertson K, Hagberg L, Zetterberg H, Gisslén M, Spudich S. Cerebrospinal fluid and neuroimaging biomarker abnormalities suggest early neurological injury in a subset of individuals during primary HIV infection. J Infect Dis. 2013; 207:1703–1712. [PubMed: 23460748]

Pornpun, V.; Gill, J.; Power, C. Differential neurologic disease burden among treated HIV/AIDS persons infected with HIV-1 clade B and clade C viruses. Conference on Retroviruses and Opportunistic Infections (CROI); 2012. Abstract: 449

Ranga U, Shankarappa R, Siddappa NB, Ramakrishna L, Nagendran R, Mahalingam M, Mahadevan A, Jayasuryan N, Satishchandra P, Shankar SK, Prasad VR. Tat protein of human immunodeficiency virus type 1 subtype C strains is a defective chemokine. J Virol. 2004; 78:2586–2590. [PubMed: 14963162]

Rao VR, Sas AR, Eugenin EA, Siddappa NB, Bimonte-Nelson H, Berman JW, Udaykumar R, Tyor WR, Prasad VR. HIV-1 clade-specific differences in the induction of neuropathogenesis. J Neurosci. 2008; 28:10010–10016. [PubMed: 18829958]

Rao VR, Neogi U, Talboom JS, Padilla L, Rahman M, Fritz-French C, Gonzalez-Ramirez S, Verma A, Wood C, Ruprecht RM, Ranga U, Azim T, Joska J, Eugenin E, Shet A, Bimonte-Nelson H, Tyor WR, Prasad VR. Clade C HIV-1 isolates circulating in Southern Africa exhibit a greater frequency of dicysteine motif-containing Tat variants than those in Southeast Asia and cause increased neurovirulence. Retrovirology. 2013; 10:61. [PubMed: 23758766]

Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci. 2001; 184:101–122. [PubMed: 11239944]

Riedel DJ, Pardo CA, McArthur J, Nath A. Therapy insight: CNS manifestations of HIV-associated immune reconstitution inflammatory syndrome. Nature Clin Pract Neurol. 2006; 2:557–565. [PubMed: 16990829]

Rotta I, Raboni SM, Ribeiro CE, Riedel M, Winhescki Mda G, Smith DM, Ellis RJ, de Almeida SM. Cerebrospinal fluid can be used for HIV genotyping when it fails in blood. Arq Neuropsiquiatr. 2014; 72:506–9. [PubMed: 25054982]

Satishchandra P, Nalini A, Gourie-Devi M, Khanna N, Santosh V, Rasvi V, Desai A, Chandramuki A, Jayakumar PN, Shankar SK. Profile of neurologic disorders associated with HIV/AIDS from Bangalore, South India (1989–96). Indian J Med Res. 2000; 111:14–23. [PubMed: 10793489]

Sheng WS, Hu S, Ni HT, Rowen TN, Lokensgard JR, Peterson PK. TNF-alpha-induced chemokine production and apoptosis in human neural precursor cells. J Leukoc Biol. 2005; 78(6):1233–41. [PubMed: 16314440]

Sherry B, Espinoza M, Manogue KR, Cerami A. Induction of the chemokine beta peptides, MIP-1 alpha and MIP-1 beta, by lipopolysaccharide is differentially regulated by immunomodulatory cytokines gamma-IFN, IL-10, IL-4, and TGF-beta. Mol Med. 1998; 4:648–657. [PubMed: 9848081]

Teja VD, Talasila SR, Vemu L. Neurologic manifestations of HIV infection: an Indian hospital-based study. AIDS Read. 2005; 15:139–143. [PubMed: 15786577]

Vittinghoff, E.; Glidden, DV.; Shiboski, SC.; McCulloch, CE. Regression Methods in Biostatistics. 2nd. NY: Springer; 2011. p. 405-408.section 10.2

Wada NI, Jacobson LP, Margolick JB, Breen EC, Macatangay B, Penugonda S, Martínez-maza O, Bream JH. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS. 2015; 29(4):463–471. [PubMed: 25630041]

Weiss JM, Nath A, Major EO, Berman JW. HIV-1 Tat induces monocyte chemoattractant protein-1-mediated monocyte transmigration across a model of the human blood-brain barrier and up-regulates CCR5 expression on human monocytes. J Immunol. 1999; 163:2953–2959. [PubMed: 10453044]

Woodman SE, Benveniste EN, Nath A, Berman JW. Human immunodeficiency virus type 1 Tat protein induces adhesion molecule expression in astrocytes. J Neurovirol. 1999; 5:678–684. [PubMed: 10602408]

Yuan L, Qiao L, Wei F, Yin J, Liu L, Ji Y, Smith D, Li N, Chen D. Cytokines in CSF correlate with HIV-associated neurocognitive disorders in the post-HAART era in China. J Neurovirol. 2013; 19:144–149. [PubMed: 23389619]

Zhang R, Miller RG, Gascon R, Champion S, Katz J, Lancero M, Narvaez A, Honrada R, Ruvalcaba D, McGrath MS. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). J Neuroimmunol. 2009; 206:121–124. [PubMed: 19013651]

Zlotnik A, Yoshie O. Chemokines: A new classification system and their role in immunity. Immunity. 2000; 12:121–127. [PubMed: 10714678]

Table 1

Demographic data, HIV disease characteristics, and hepatitis C virus co-infection by HIV subtype and by study group.

	Subtype B n = 27	**Subtype C n = 25**	**HIV- n = 19**	**P-value B vs. C**	**P-value, HIV+ vs. HIV−**
Age, y, median (IQR)	44 (37,50)	43 (35, 47)	41 (38, 48)	0.33	0.76
Gender				0.419	0.073
- Male, n (%)	14 (52)	10 (40)	14 (74)		
- Female, n (%)	13 (48)	15 (60)	5 (26)		
AIDS, n (%)	21 (78)	18 (72)	-	0.75	-
ART, n (%)	24 (89)	16 (64)	-	0.049	-
Current CD4[1]	457 (255, 607)	372 (196, 367)	-	0.19	-
Nadir CD4[1]	83 (42, 232)	175 (31, 347)	-	0.25	-
Duration of infection[1](months)	87 (76, 130)	79 (26, 136)	-	0.47	-
HCV[2] (%)	6 (22)	2 (8)	0	0.25	-
Plasma $\log_{10}$ viral load	1.70 (1.70, 1.81)	2.92 (1.70, 3.77)	-	0.008	-
Plasma viral load <50, n (%)	20 (74)	8 (32)	-	0.005	-

[1]Median (IQR)

[2]Hepatitis C virus (HCV) serostatus was assessed using an HCV antibody test (Abbott-Architect).

Table 2

Cerebrospinal fluid biochemical, cytological, and virological characteristics by study group

	Subtype B n = 27	Subtype C n = 25	HIV- n = 19	P-value, Subtypes B vs. C	P-value, HIV+ vs. HIV−
Glucose[1,2] mg/dL	63 (54, 66)	56 (52, 59)	63 (59, 71)	0.004	0.001
Total Protein[1] mg/dL	42 (35, 48)	40 (29, 46)	30 (26, 38)	0.41	0.004
WBC[1] cells/mm^3	1.6 (0.3, 4,9)	3.4 (0.6, 11)	2 (1.0, 2.5)	0.23	0.63
WBC > 5 cell/mm^3	6 (22%)	8 (32%)	0	0.43	0.015
Lactic Acid[1] mmol/L	1.7 (1.4, 1.9)	1.7 (1.6, 1.8)	-	0.76	-
RBC[1] cells/mm^3	1.0 (0.0, 24)	0.6 (0.0, 22)	2.0 (1.0, 4.0)	0.75	0.72
CSF HIV RNA Log10[1]	1.7 (1.7, 2.2)	2.2 (1.7, 2.9)	-	0.06	-
Log CSF HIV RNA <1.7	16 (59%)	09 (36%)	-	0.11	-
HIV RNA CSF > blood	6 (22%)	4 (16%)	-	0.73	-
Albumin[1] mg/dL	25 (19; 31)	22 (14; 29)	18 (15, 24)	0.57	0.03
QAlb[1]	0.008 (0.006; 0.011)	0.006 (0.004; 0.009)	0.005 (0.004; 0.006)	0.50	<0.0001

Glucose: mg/dL; Total protein: mg/dL; WBC: cell/mm^3; lactic acid: mmol/L; VL: viral load; albumin: mg/dL

[1]Median (IQR);

Table 3

HIV-positive and HIV-negative levels of cellular chemotaxis and inflammatory biomarkers in cerebrospinal fluid and serum. Significant differences are in **bold** typeface.

	CSF HIV+	CSF HIV−	Diff (95% CI)[1]	p	Serum HIV+	Serum HIV−	Diff (95% CI)[1]	p
Cytokines								
TNFα	**0.31 (0.51)**	**0.04 (0.24)**	**0.59 (0.2, 0.98)**	**0.004**	**0.94 (0.19)**	**0.79 (0.18)**	**0.82 (0.29, 1.34)**	**0.003**
IFNy	**0.29 (0.44)**	**0.07 (0.29)**	**0.53 (0.08, 0.97)**	**0.022**	**0.25 (0.46)**	**0.56 (0.47)**	**−0.67 (−1.22, −0.12)**	**0.019**
IL1β	−0.10 (0.03)	−0.10 (0.00)	−0.28 (−0.6, 0.04)	0.088	−0.09(0.08)	−0.02(0.18)	−0.52 (−1.28, 0.24)	0.170
IL-2	0.02 (0.09)	0	0.27 (−0.05, 0.59)	0.100	0.08 (0.36)	0.08 (0.19)	0 (−0.41, 0.42)	0.990
IL-4	0.65 (0.00)	0.65 (0.00)	0 (0.0)	1.000	0.66 (0.15)	0.77 (0.39)	−0.44 (−1.25, 0.37)	0.270
IL-6	0.14 (0.32)	0.09 (0.27)	0.18 (−0.32, 0.68)	0.470	0.06(0.30)	0.08(0.26)	−0.05 (−0.55,0.46)	0.850
IL-7	**0.28 (0.21)**	**0.17 (0.14)**	**0.59 (0.14, 1.03)**	**0.011**	0.70 (0.35)	0.65 (0.28)	0.13 (−0.36, 0.62)	0.600
IL-10	**0.26 (0.43)**	**0.04 (0.0)**	**0.60 (0.28, 0.93)**	**0.0005**	0.31 (0.42)	0.30 (0.40)	0.02 (−0.52, 0.55)	0.950
Chemokines								
IP-10	**3.29 (0.83)**	**2.98 (0.20)**	**0.43 (0.08, 0.77)**	**0.015**	**2.98 (0.31)**	**2.57(0.20)**	**1.42 (0.98, 1.87)**	**<0.0001**
MCP-1	**3.15 (0.20)**	**3.05 (0.12)**	**0.57 (0.14, 1.0)**	**0.010**	2.73(0.22)	2.63(0.22)	0.45 (−0.1, 1)	0.100
MIP-1α	**1.09 (0.14)**	**1.01 (0.06)**	**0.65 (0.26, 1.04)**	**0.001**	0.65(0.32)	0.64(0.29)	0.03 (−0.49, 0.55)	0.900
MIP-1β	1.06 (0.29)	0.95 (0.30)	0.38 (−0.18, 0.93)	0.170	1.71(0.28)	1.72(0.19)	−0.04 (−0.49, 0.42)	0.880
RANTES	0.60 (0.64)	0.37 (0.57)	0.37 (−0.15, 0.89)	0.150	**4.49(0.26)**	**4.75(0.20)**	**−1.04 (−1.51, −0.56)**	**<0.0001**

Values are $\log_{10}$ transformed pg/mL, presented as mean (SD).

[1] Groups differences presented as Cohen´s d effect sizes and 95% confidence intervals.

Table 4

HIV-1 subtype B and C- adjusted levels of cellular chemotaxis and Inflammatory biomarkers in cerebrospinal fluid and serum. None of the differences was statistically significant.

	CSF				Serum			
	Subtype B	Subtype C	Diff (95% CI)[1]	p[2]	Subtype B	Subtype C	Diff (95% CI)[1]	p[2]
Cytokines								
TNFα	0.25 (0.47)	0.36 (0.54)	−0.07(−0.69,0.55)	0.83	0.97 (0.15)	0.92 (0.23)	0.46 (−0.16, 1.08)	0.14
IFN y	0.28 (0.43)	0.30 (0.46)	0.12 (−0.5,0.74)	0.70	0.18 (0.38)	0.32 (0.53)	−0.53 (−1.15, 0.09)	0.09
IL1β	−0.11(0.04)	−0.10 (0.02)	−0.18 (−0.8,0.44)	0.56	−0.07 (0.12)	−0.10 (0.00)	0.50 (−0.12, 1.12)	0.11
IL-2	0	0.04 (0.13)	−0.45 (−1.07, 0.17)	0.15	0.06 (0.19)	0.11 (0.48)	−0.25 (−0.87, 0.37)	0.42
IL-4	0.64 (0.00)	0.65 (0.00)	−0.20(−0.82, 0.42)	0.51	0.64 (0.12)	0.69 (0.17)	−0.50 (−1.12, 0.12)	0.11
IL-6	0.15 (0.36)	0.14 (0.28)	0.03 (−0.59, 0.65)	0.92	0.06 (0.27)	0.07 (0.34)	0.047 (−0.57, 0.67)	0.88
IL-7	0.28 (0.21)	0.29 (0.22)	0.20 (−0.42, 0.82)	0.52	0.66 (0.39)	0.74 (0.30)	−0.448 (−1.07, 0.17)	0.15
IL-10	0.22 (0.40)	0.31 (0.46)	−0.15 (−0.77, 0.47)	0.63	0.33 (0.47)	0.29 (0.36)	0.16 (−0.46, 0.78)	0.60
Chemokines								
IP-10	3.21 (0.71)	3.37 (0.95)	−0.06 (−0.68, 0.56)	0.86	2.90 (0.33)	3.05 (0.27)	−0.06 (−0.68, 0.56)	0.85
MCP-1	3.15 (0.18)	3.15 (0.21)	0.02 (−0.6, 0.64)	0.96	2.77 (0.19)	2.69 (0.24)	0.11 (−0.51, 0.73)	0.73
MIP-1α	1.08 (0.12)	1.11 (0.16)	−0.04 (−0.66, 0.58)	0.89	0.64 (0.33)	0.66 (0.32)	−0.203 (−0.82, 0.42)	0.51
MIP-1β	1.07 (0.23)	1.05 (0.36)	−0.13 (−0.75, 0.49)	0.67	1.78 (0.23)	1.64 (0.31)	0.29 (−0.34, 0.90)	0.37
RANTES	0.52 (0.48)	0.69 (0.77)	−0.21 (−0.83, 0.41)	0.50	4.49 (0.30)	4.50 (0.22)	−0.02 (−0.64, 0.60)	0.96

[1] Groups differences presented as Cohen's d effect sizes and 95% confidence intervals.

[2] Adjusted for plasma and HIV viral load suppression, and CD4 nadir.