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Blood-CSF barrier and compartmentalization of CNS cellular immune response in HIV infection

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ABSTRACT

HIV infection is persistent in the CNS, to evaluate the compartmentalization of the CNS immune response to HIV, we compared soluble markers of cellular immunity in the blood and CSF among HIV − (n = 19) and HIV + (n = 68), as well as among HIV participants without or with CSF pleocytosis. Dysfunction of the blood cerebrospinal fluid barrier (BCSFB) was common in HIV participants. CSF levels of TNFα, IFNγ, IL-2, IL-6, IL-7, IL-10, IP-10, MIP-1α, MIP-1β, and RANTES were significantly higher in participants with CSF pleocytosis (P < 0.05); serum levels of these biomarkers were comparable. The CNS immune response is compartmentalized, and remains so despite the BCSFB dysfunction during HIV infection; it is markedly reduced by virology suppression, although BCSFB dysfunction persists on this subgroup.

1. Introduction

The CNS barriers, i.e. blood-brain barrier (BBB) and the blood-CSF barrier (BCSFB) are highly selective physiological barriers that restrict the passage of macromolecules from circulating blood to the CNS. These barriers protect neurons from constant changes in the concentration of blood constituents without restricting the transport of nutrients and metabolic products to and from the brain, respectively, as well as the bidirectional communication with the immune system (de Vries et al., 1997; Ballabh et al., 2004; Hamilton et al., 2007; Stamatovic et al., 2008; Abbott et al., 2010; Banks, 2015). Reductions in CSF production, release, and flow rate impair the BCSFB function, thereby increasing the ratio of CSF albumin to serum albumin (albumin quotient Qab) (Kuehne et al., 2013).

In recent years, there has been a resurgence of interest in the physiology of the brain barriers, which may play critical roles in a wide range of neurological disorders, including those associated with HIV infection (Saunders et al., 2008). Studies conducted prior to the development of highly active anti-retroviral therapy demonstrated that dysfunction of the BCSFB was common in HIV participants, particularly in those with increased CSF white blood cell (WBC) count (pleocytosis) (Marshall et al., 1988; Petito and Cash, 1992). More recent studies also found evidence of BCSFB dysfunction (Andersson et al., 2001; Calcagno et al., 2014), which can occur early in infection, and persist even with highly active anti-retroviral therapy (Calcagno et al., 2015).

HIV is a systemic infection, of which the nervous system is an important component (Brew et al., 1997). Indeed, HIV is highly neurotropic and neurovirulent, and causes persistent infection in the CNS. Invasion of the CNS during acute HIV infection (Resnick et al., 1988; Spudich et al., 2011; Valcour et al., 2013) initiates an inflammatory cascade that eventually compartmentalizes the CNS immune response even with anti-retroviral therapy. Thus we hypothesize that the inflammatory response in HIV infection is stronger in CNS than peripheral blood, characterizing compartmentalization of CNS cellular immune response; despite the dysfunction of the BCSFB during HIV infection. By compartmentalization of immune responses we mean evidence of immune activity, including production of signaling molecules such as chemokines and interleukins, that is CNS specific and would be missed by studying only plasma or serum.

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To evaluate the compartmentalization of the CNS immune response to HIV, we compared soluble markers of cellular immunity in the blood and CSF among uninfected and HIV-infected individuals, as well as among HIV participants with or without CSF pleocytosis. We selected biomarkers on the basis of their roles in cellular immunity, neuroinflammation, chemotaxis, and CNS HIV pathobiology (Nath, 1999; Genis et al., 1992; King et al., 2006; Campbell et al., 2007a; Abbas and Herbein, 2013). We also investigated the BCSFB function, and identified the origin of CSF biomarkers.

We anticipated that the data would provide important pathophysiological insights into the neurological impact of HIV and the specific immune response in the CNS. This study extends previous studies (Price et al., 2013; Peterson et al., 2014) by examining cellular immunity in the CSF and serum of participants infected with non-B HIV subtypes, noting that HIV-infected samples we tested were collected from the same geographic area. Indeed, previous studies focused almost exclusively on the effects of HIV-1 subtype B on inflammatory and chemotaxis biomarkers (Genis et al., 1992; Cinque et al., 2007; Brew and Letendre, 2008; Hagberg et al., 2010; Yuan et al., 2013).

2. Materials and methods

This study was a cross-sectional survey of stored CSF and serum samples, and was approved by institutional review boards at University of California San Diego, Hospital de Clínicas-UFPR in Brazil, and Brazil National IRB (CONEP). Participants and methods were described previously (de Almeida et al., 2013, 2016).

2.1. Subjects

A total of 87 paired CSF and serum samples were analyzed. Demographic characteristics, HIV status, and co-infections, if any, are summarized in Table 1 for HIV-positive (n = 68) and HIV-negative (n = 19) volunteers.

Table 1

<table>
<thead>
<tr>
<th>HIV status and treatment</th>
<th>HIV + (n = 68)</th>
<th>HIV − (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>43 (35; 48)</td>
<td>41 (38; 50)</td>
<td>0.91</td>
</tr>
<tr>
<td>Education, years</td>
<td>8 (5; 11)</td>
<td>12 (11; 15)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gender, n male (%)</td>
<td>33 (49)</td>
<td>14 (74)</td>
<td>0.07</td>
</tr>
<tr>
<td>HIV status and treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS, n (%)</td>
<td>55 (81)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Duration of infection, months</td>
<td>99 (29; 140)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Current CD4</td>
<td>369 (201; 534)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log plasma HIV RNAa</td>
<td>1.7 (1.7; 3.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Plasma HIV RNA &lt; 50 copies/ml, n (%)</td>
<td>38 (56)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log CSF HIV RNA</td>
<td>1.7 (1.7; 2.8)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CSF HIV RNA &lt; 50 copies/ml, n (%)</td>
<td>36 (53)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>On CARTb, n (%)</td>
<td>55 (81)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CPEc</td>
<td>8 (6; 9)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Adherenced, n (%)</td>
<td>51 (93)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCVc, n (%)</td>
<td>12 (18)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Log plasma HCV RNA, n (%)</td>
<td>2.9 (1.7; 5.9)</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

Participants co-infected with HCV were not on treatment with interferon-gamma. Data are median (IQR) or number of cases (%).

a Plasma viral load, log_{10} copies/mL.

b CART, combination anti-retroviral therapy.

c Anti-retroviral treatment adherence was evaluated using AIDS clinical trial (ACTG) adherence questionnaire (4-day recall).

d Hepatitis C virus (HCV) status was assessed by antibody testing (Abbott-Architect).

2.1.1. HIV participants

HIV participants were recruited at Hospital de Clínicas, Universidade Federal do Paraná, Curitiba, Paraná, Brazil. Individuals with opportunistic CNS infections were excluded. All volunteers provided blood and CSF samples, and underwent serological testing to confirm HIV status before enrollment, in accordance with guidelines published by the Brazilian Ministry of Health (2009). For participants with clinically resistant infection, the infecting HIV strain was genotyped using pol sequences, while env sequences were used for all other participants. Genotyping indicated that 27 individuals were infected with HIV subtype B, and 40 with non-B HIV subtypes (C-26, BF-10, BC-1, CF-1, and F-2). In one participant, the HIV subtype could not be determined.

2.1.2. Uninfected controls

As lumbar punctures could not be performed in uninfected volunteers in Brazil, we recruited a control group of 19 age-matched HIV-negative individuals at HIV Neurobehavioral Research Center, University of California San Diego. These volunteers were free of neurological comorbidities, and tested negative on serological tests for hepatitis C virus and syphilis. The neurochemical criteria for inclusion into the control group were CSF WBC count ≤5 cells/mm³, CSF total protein ≤45 mg/dL, and CSF glucose ≥55 mg/dL.

2.2. Laboratory methods

Lumbar punctures were performed aseptically using an atraumatic spinal needle. CSF total protein and glucose were quantified by benzethonium chloride and hexoquinase/G-6-PDH (Architect-Abbott, IL), respectively. Total WBC/mm³ was determined in fresh, uncentrifuged CSF by manual counting in a Fuchs-Rosenthal chamber. CSF pleocytosis was defined as WBC >5 cells/mm³. For differential leukocyte counts, CSF samples were concentrated by Shandon Cytospin (Pittsburgh, USA), mounted, and stained by May-Grünwald-Giemsa technique. Aliquots of CSF and serum were refrozen and stored at -80 °C for subsequent batch testing for cytokines and chemokines.

2.2.1. Clinical laboratory parameters

HIV RNA levels in serum and CSF were quantified by branched DNA assay (Siemens) with nominal limit of detection 50 copies/mL. CD4 counts were quantified by flow cytometry (FACSCalibur-Multitest), while nadir CD4 was extracted from medical records.

2.2.1.1. CSF and serum biomarkers.

RANTES was quantified by high-sensitivity enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN), TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-10, MCP-1, MIP-1α, and IP-10 were quantified by multiplex bead suspension array immunosays (EMD Millipore, Billerica, MA), in which primary antibodies are immobilized to dyed fluorescent microspheres. The analytic sensitivity of these assays ranged between 0.1 and 10.1 pg/mL. All samples were assayed concurrently in duplicate according to manufacturers’ instructions.

2.2.1.2. Blood-CSF barrier function.

The functional integrity of the blood-CSF barrier was then assessed by the CSF albumin/serum albumin quotient, which was calculated as QAlb = AlbCSF∕Albserum. The upper limit of the reference range for QAlb is age-dependent, was calculated for each participant according to the equation: 4 + age in years∕15 (Reiber and Peter, 2001). The albumin and IgG hyperbolic function was calculated using the Reibergram plot (Reiber, 1995). Albumin leakage across the blood-brain barrier over 24 h was also calculated (Tourtellotte et al., 1989).
2.3. Data analyses

Demographic data, HIV disease characteristics, and CSF biochemical, cytological, and virological measures were compared between groups using independent samples t-tests 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 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.

In order to detect the source of the biomarkers in CSF was calculated the coefficient of variation (CV) for each biomarker in CSF and serum in the normal control group; to the evaluation of the inter-individual variation. The comparison of biological variation of molecule concentration can be used to compare functionally connected compartments of the same group; if we could expect variation of propagation for molecules due to the passage from one compartment to the other (Kuehne et al., 2013).

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 compared between the HIV-positive and HIV-negative control groups as well as between the HIV-positive samples with pleocytosis in the CSF and HIV-positive samples with a normal CSF WBC count. We used a multivariable linear regression (adjusted analysis), controlling for plasma HIV viral load suppression, and nadir CD4 count, which has been shown in previous studies to be associated with increased soluble biomarkers of inflammation and chemotaxis in HIV (Cisslen et al., 1994; Mooney et al., 2015; Noel et al., 2014). In a second moment results were adjusted for QAb for the comparison of groups HIV+ and HIV−; and QAb in addition to suppressed plasma and CSF viral load, and CD4 nadir for the comparison of the groups with and without CSF pleocytosis.

Correlations between variables were calculated using Spearman’s rank-order correlation. 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.

3. Results

As shown in Table 1, ART was prescribed to 55 (81%) of the HIV+ participants. The most frequent ART regimen (33 participants; 60%) contained a ritonavir-boosted HIV protease inhibitor (PI) plus two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI; NtRTI); 18 participants (33%) received a non-nucleoside RT inhibitor (NNRTI) with 2 NRTIs, and 4 (7%) participants received other regimen types. Darunavir was not available in Brazil during the time that these samples were collected. Because of this the Brazilian National HIV Therapy protocol recommended that patients with resistance receive a regimen of 2 NRTIs, and 4 (7%) participants received other regimen types.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>HIV+ (n = 68)</th>
<th>HIV− (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC cells/mm$^3$</td>
<td>2.1 (0.6; 7.2)</td>
<td>2 (1.25)</td>
<td>0.4024</td>
</tr>
<tr>
<td>WBC count</td>
<td>20 (29%)</td>
<td>0</td>
<td>-0.0001</td>
</tr>
<tr>
<td>&gt;5 cells/mm$^3$</td>
<td>57 (53; 62)</td>
<td>63 (59; 71)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Total protein, mg/dL</td>
<td>40 (32; 46)</td>
<td>30 (26; 38)</td>
<td>0.0026</td>
</tr>
<tr>
<td>Total protein &gt;45 mg/dL</td>
<td>20 (29%)</td>
<td>0</td>
<td>0.0048</td>
</tr>
<tr>
<td>Albumin, mg/dL</td>
<td>22.4 (16.4; 28.9)</td>
<td>18 (15; 24)</td>
<td>0.019</td>
</tr>
<tr>
<td>Albumin quotient, QAb</td>
<td>0.0064 (0.0049; 0.005)</td>
<td>0.0005 (0.0014; 0.0023)</td>
<td>0.023</td>
</tr>
<tr>
<td>Lactic acid, mmol/L</td>
<td>1.6 (1.5; 1.8)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>RBC cells/mm$^3$</td>
<td>0.5 (0; 7.5)</td>
<td>2.0 (1.0; 4.0)</td>
<td>0.3993</td>
</tr>
<tr>
<td>Viral load &lt;50</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HIV RNA CSF &gt; blood</td>
<td>12 (18%)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**CSF glucose was significantly higher in HIV-negative volunteers than in HIV participants, although levels in both groups were below reference range. Data are median (IQR) or number of cases (%).**

direct CSF smear, culture and latex agglutination tests for C. neoformans capsular antigen; Ziehl smear, culture and PCR for M. tuberculosis; and PCR for HSV, enterovirus, V2V, CMV, HHV6, HHV7, EBV and JC virus.

3.2. Inflammatory biomarkers

3.2.1. Comparison between HIV-positive and HIV-negative groups

Cytokine and chemokine levels are listed in Table 3. In CSF, 9 of 13 cytokines and chemokines were significantly elevated (P < 0.05) in HIV participants than in uninfected controls. Contrast, only 4 of 13 markers were significantly elevated in serum (Table 3).

In unadjusted regression models, CSF levels of the cytokines TNFα, IFNγ, IL-1β, IL-2, IL-7, and IL-10 were significantly elevated in HIV+ participants than in uninfected individuals. In contrast, only TNFα and IFNγ were significantly elevated in serum. CSF levels of the chemokines IP-10, MCP-1, and MIP-1α were also significantly elevated in HIV participants than in uninfected controls, while IP-10 and RANTES were significantly elevated in serum. Standardized differences in CSF between HIV-positive and HIV-negative subjects (effect sizes) were larger, on average, than differences in serum between the two groups (Table 3). Adjusted analysis for CSF/serum albumin quotient (Q alb.) there was no significant difference between the groups for all cytokines and chemokines measured (P = 0.05).

Collectively, the data indicate that the cellular immune response differs qualitatively between CSF and serum, as more types of cytokines and chemokines were stimulated in CSF than in serum. Also levels of some cytokines and chemokines were also higher in CSF than in serum, clearly indicating intrathecal synthesis (Table 3). Hence, we conclude that the immunological response is stronger in CSF than in serum.

In a subgroup analysis of plasma virologically suppressed individuals (n = 37), MIP-1β was significantly elevated compared to HIV negative subjects (P = 0.027).

3.2.2. Comparison between HIV participants with or without CSF pleocytosis

CSF and serum cytokines and chemokines in HIV participants with or without CSF pleocytosis are summarized in Table 4. After adjusting for CD4 nadir and viral load suppression in plasma and CSF, most cytokines, including TNFα, IFNγ, IL-1β, IL-2, IL-6, IL-7, and IL-10, were elevated, along with the chemokines IP10, MIP-1α, MIP-1β and RANTES, in the CSF of HIV participants with CSF pleocytosis than in participants without pleocytosis. Additionally, CSF levels of MCP-1 tended to be higher in participants with pleocytosis, although differences were not statistically significant. In contrast, serum cytokines and chemokines were comparable. These findings are consistent with the idea that the immune response to HIV differs between blood and CNS.
We note that in participants with pleocytosis, the cytokines IFNγ, IL-1β, IL-2, IL-6, and IL-10, as well as the chemokines IP-10, MCP-1, and MIP-1α, were higher in CSF than in serum, indicating intrathecal synthesis (Table 4).

Finally, statistically significant differences in TNFα, IL-10, IP-10 (all \( P < 0.0001 \)), MIP-1α (\( P = 0.0069 \)), MIP-1β (\( P = 0.014 \)), and RANTES (\( P = 0.00015 \)) persisted after adjusting for \( Q_{AB} \), CD4 nadir, and viral load suppression in plasma and CSF.

### 3.3. Origin of CSF biomarkers

In uninfected controls, the coefficients of variation for IFNγ, IL-7, and MCP-1 were much smaller in CSF (0.81, 0.32, and 0.24, respectively) than in serum (1.25, 0.57, and 0.49, respectively), suggesting that these molecules are not derived from blood, but from the brain or the leptomeninges (Kuehne et al., 2013). In contrast, we found that the coefficient of variation for albumin was higher in CSF (0.299) than in serum (0.063), in line with published data (Kuehne et al., 2013). Indeed, a molecule that passes from blood to CSF should have a larger inter-individual variation in CSF than in blood due to variability in CSF flow and transport across barriers (Reiber et al., 2012). Thus, albumin can be used as a positive control for this analysis, because CSF albumin is exclusively blood-derived. For all other biomarkers (IL-6, TNFα, IP-10, MIP-1α, and RANTES) the variability was higher in CSF than in serum. These results, however, are inconclusive, and may indicate either transport from blood or limited regulatory control of release from the brain (Reiber et al., 2012). For IL-1β, IL-2, IL-4, and IL-10, the coefficient of variation is smaller in CSF than in blood, although levels were below the assay detection limits in the uninfected volunteers.

In HIV participants, the levels of the cytokines IL-6 and the chemokines IP-10, MCP-1, and MIP-1α were higher in CSF than in serum, also indicating intrathecal synthesis (Table 3).

### 3.4. Blood-CSF barrier function

\( Q_{AB} \) was significantly higher in HIV participants than in uninfected controls, and was measured to be \( 6.4 \times 10^{-3} \) (4.9 \( \times 10^{-3} \), 9.7 \( \times 10^{-3} \)) in the former and 5 \( \times 10^{-3} \) (4 \( \times 10^{-3} \), 6 \( \times 10^{-3} \)) in the latter (Table 2; Fig. 1; \( P = 0.0023 \)). In HIV participants with and without CSF pleocytosis, \( Q_{AB} \) was determined to be 7.9 \( \times 10^{-3} \) (6.3 \( \times 10^{-3} \), 11.6 \( \times 10^{-3} \)) and 6.1 \( \times 10^{-3} \) (4.5 \( \times 10^{-3} \), 9.2 \( \times 10^{-3} \)), respectively (\( P = 0.014 \)). Based on age-dependent reference values, there was BCSFB dysfunction in 30% (44%) HIV-positive individuals, but not in uninfected controls (\( P = 0.0002 \)). BCSFB dysfunction was observed in 13 (65%) HIV participants with CSF pleocytosis, as well as in 17 (35%) participants with normal white blood cell count in the CSF (\( P = 0.0033 \)). \( Q_{AB} \) and albumin Tourtellotte’s formula are summarized in Fig. 1A and B, respectively; the Reibergram plot is shown in Fig. 2.

In a subgroup analysis of plasma virologically suppressed individuals (\( n = 37 \)), \( Q_{AB} \) and albumin Tourtellotte’s formula remained significantly elevated compared to HIV negative subjects (\( P = 0.001 \) and 0.002).

In HIV participants, the CSF WBC count was weakly but positively correlated with \( Q_{AB} \) (\( r_s = 0.27 \); \( P = 0.0275 \)), indicating that CSF WBC is not a marker of BCSFB function. However, there was strong correlation between CSF total protein and \( Q_{AB} \) (\( r_s = 0.8 \); \( P < 0.0001 \)).

### 3.5. Correlation of CSF inflammatory biomarkers with CSF flow rate; CSF or plasma HIV RNA

\( Q_{AB} \), a measure of CSF flow rate, was moderately and positively correlated with CSF for the majority of cytokines and chemokines studied on the HIV+ participants, \( r_s \) and \( P \) values ranged from \( r_s = 0.289 \) (\( P = 0.017 \)) to \( r_s = 0.483 \) (\( P < 0.0001 \)), for IL-7 and TNFα respectively. The correlation was not significant for IL-1β, IL-4, and IL-10. Similar results were obtained when biomarkers were tested for correlation with albumin Tourtellotte’s formula. These correlations may explain why...
incorporation of $Q_{ Alb}$ into regression models diminishes the significance of a majority of biomarkers. Hence, we conclude that BCSFB dysfunction critically affects and accounts for levels of inflammatory biomarkers in CSF.

CSF and plasma viral load are important as HIV antigen load may drive cytokine responses. Indeed, CSF HIV RNA was moderately and positively correlated with levels of CSF IFNγ ($r = 0.472$, $P < 0.0001$); IL-10 ($r = 0.380$, $P = 0.0013$); IL-6 ($r = 0.415$, $P = 0.0004$); IP-10 ($r = 0.518$, $P < 0.0001$); TNFα ($r = 0.618$, $P < 0.0001$); IP-10 ($r = 0.562$, $P < 0.0001$); MCP-1 ($r = 0.333$, $P = 0.0055$); and RANTES ($r = 0.488$, $P < 0.0001$); Plasma HIV RNA was moderately and positively correlated with CSF IFNγ ($r = 0.369$, $P = 0.002$); IL-6 ($r = 0.263$, $P = 0.031$); IL-6 ($r = 0.464$, $P < 0.0001$); TNFα ($r = 0.418$, $P = 0.0004$); IP-10 ($r = 0.474$, $P < 0.0001$); MCP-1 ($r = 0.397$, $P = 0.0008$); RANTES ($r = 0.284$, $P = 0.019$). The majority of cases (26; 81%) with detectable HIV CSF viral load also had detectable HIV RNA in blood. To account for this our statistical analysis adjusted for blood viral load.

**4. Discussion**

In this cross-sectional analysis, unadjusted regression models detected differences in biomarkers in CSF, but not in serum, between HIV-positive and HIV-negative volunteers. This result strongly suggested compartmentalization of the CNS inflammatory response to HIV. In addition, we observed dysfunction of the BCSFB in HIV participants, especially among participants with CSF pleocytosis. The functional integrity of this barrier is generally defined by diffusion and CSF flow rate, the latter being a function of CSF production and release, and is typically assessed by measuring CSF albumin. Dysfunction of the BCSFB is generally due to a decrease in CSF flow rate, rather than structural disruption (Reiber et al., 2012).

In HIV participants, CSF levels of a majority of inflammation biomarkers were positively correlated with CSF/serum albumin ratio. Consequently, incorporation of this ratio into regression models diminished the differences between HIV-positive and HIV-negative CSF, and between HIV participants with or without CSF pleocytosis. These observations indicate that CSF flow rate plays a critical role in CNS inflammation, presumably by facilitating influx of inflammatory molecules, white blood cells, HIV particles, and HIV proteins from peripheral blood, and thereby exacerbating and sustaining cellular immunity and inflammation in the CNS. If the BCSFB dysfunction is cause or consequence of the inflammation can't be answered by this study. Presumably probably both process are involved, this observation is shared by others authors (Saunders et al., 2008). CNS barriers actively regulate brain homeostasis, and specifically respond to events in peripheral tissues and in the brain parenchyma. Indeed, these activities should be considered to fully characterize CNS diseases. Consequently, there has been a resurgence of interest in the possibility that barrier dysfunction may be involved in different neurological disorders (Saunders et al., 2008).

Our data indicate that in HIV participants, cytokine levels were altered more drastically in the CSF than in the serum. Although this phenomenon was observed for both pro-inflammatory (TNFα and IFNγ) and anti-inflammatory (IL-10) cytokines, the effect was stronger in the former. Levels of IL-7, an important inducer of autoimmune reactions that elicit a strong response to IL-17, i.e., Th-17 response were also elevated in CSF. In contrast, only pro-inflammatory cytokines (TNFα and IFNγ) were altered in the serum. Taken together, the data support the notion that the CNS is a primary site of HIV infection, and are consistent with the hypothesis that the CNS reacts immunologically when stimulated appropriately, although the system is considered immunologically privileged (Barker and Billingham, 1977; Kreutzberg, 1996).

Although CNS HIV infection originates from viral and immune components transported into the CNS, the infection eventually becomes...
“compartmentalized” and acquires characteristics distinct from those observed in the blood. Thus, viral load and levels of immune components may be determined by independent processes in the CSF and blood (Price et al., 2013). Accordingly, a previous study demonstrated that inflammatory reactions in the CSF and serum are comparable during primary HIV infection, but eventually diverge as the infection progresses and as HIV-associated neurocognitive disorders develop. In addition, persistent CSF and blood inflammation despite HIV suppression suggests incomplete control of systemic and CNS infection (Peterson et al., 2014).

We note that all chemokines stimulated by HIV infection are induced via IFNy, for instance, IP-10 and MCP-1. Similarly, RANTES, was almost twice as high in the CSF in HIV participants, although this difference was not statistically significant. MIP-1x is also elevated, although this chemokine is down-regulated by IFNy in vitro (Sherry et al., 1998). In addition, IP-10 and RANTES levels were elevated in the serum of HIV participants.

The origins of most CSF biomarkers tested in this study are unknown, and tracing such origins may help elucidate the dynamics of these biomarkers. CSF molecules can be derived exclusively from blood, brain parenchyma or from the leptomeninges (Kuehne et al., 2013; Reiber, 2001; Reiber et al., 2012). Our data indicate that IL-6, IL-7, IFNy, MCP-1, MIP-1x, and IP-10 are derived from intrathecal synthesis, either because the concentration was higher in CSF than in serum (IL-6, IP-10, MCP-1, and MIP-1x), or because the coefficient of variation was smaller in CSF than in serum (IFNy, IL-7, and MCP-1). The latter result suggests that inter-individual variations in CSF biomarkers do not depend on serum concentration. Thus, we believe that CSF/serum quotients for these biomarkers primarily derived from the brain are not informative (Reiber, 2001, 2003). However, for proteins derived primarily from blood, the CSF/serum quotients may indeed be informative. We note that coefficients of variation for molecules derived from blood increase with transport into CSF because of variability in barrier trafficking and CSF flow rate (Kuehne et al., 2013).

Further, we note that several proteins we analyzed are of small size, including IFNy (17 kDa), IL-1β (17.5 kDa), IL-2 (15 kDa), IL-4 (17 kDa), IL-6 (25 kDa), IL-7 (17 kDa), IL-10 (20 kDa), TNFα (18 kDa), IP-10 (8.7 kDa), MCP-1 (8 kDa), MIP-1x (20 kDa), MIP-1β (8–14 kDa), and RANTES (7.8 kDa). Hence, these proteins may also cross the blood-CSF barrier independent of its integrity, as diffusion through an intact barrier may occur along a concentration gradient (Reiber, 2001, 2003; Reiber et al., 2012), although such diffusion might be affected by changes in CSF flow rate. Our data are in line with these observations, as CSF levels of all cytokines and chemokines were obtained from HIV participants, and 19 from uninfected controls. Nevertheless, we detected significant medium- and large-effect sizes between infected and uninfected volunteers, and between HIV participants with or without CSF pleocytosis. These effects are expressed in terms of Cohen’s d and 95% confidence intervals. Notably, Cohen’s effect sizes were mostly large in comparisons between HIV participants with or without pleocytosis. Furthermore, the samples included participants infected with non-B HIV subtypes, in contrast to previously published papers. Although HIV-1 subtypes differences on biomarkers were not the aim of this study, comparisons on the same panel of inflammatory biomarkers between HIV-1 subtype B and C were published on a previous paper, we note that inflammatory biomarkers were found to be

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comparable between participants infected with these subtypes (de Almeida et al., 2016).

Finally, previous papers did not fully integrate multiple results from studies of the BCSFB during HIV infection, and did not reach the conclusion that a dysfunctional barrier helps to maintain and increase the divergence of the immune response in CSF and blood. Nevertheless, this study is not without limitations, and we emphasize that results are cross-sectional, and not longitudinal.

As conclusion, our findings indicate that despite dysfunction of the BCSFB, cellular immune response remains highly compartmentalized. Thus markers of cellular immune responses to HIV in CSF are distinct from those in serum. Furthermore, chemokines and cytokines were frequently elevated in the CSF of HIV participants, in comparison to uninfected controls. CSF chemokines and cytokines correlated with CSF and plasma HIV RNA, suggesting that HIV antigen load may drive cytokine responses. These results support the notion that the CNS behaves as a separate immunologic compartment. This compartmentalized immune response is markedly reduced by vireology suppression, although BCSFB dysfunction persists on this subgroup. Finally, we found that dysfunction of the BCSFB critically contributes to the strong CNS inflammatory response to HIV, presumably by allowing transport of WBC, serum molecules, HIV particles, and HIV proteins into the CNS.

Abbreviations

BBB: blood-brain barrier
BCSFB: blood-CSF barrier
CART: combination anti-retroviral therapy
CPE: CNS anti-retroviral penetration effectiveness
IFN: interferon
IL: interleukin
IP-10: interferon-gamma-induced protein 10
QAlb: albumin quotient
MCP: monocyte chemoattractant protein
MIP: macrophage inflammatory protein
RANTES: regulated on activation, normal T-cell expressed and secreted
TNF: tumor necrosis factor
WBC: white blood cell

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