Cerebrospinal fluid and plasma lipopolysaccharide (LPS) levels in HIV-1 infection and associations with inflammation, blood-brain barrier permeability and neuronal injury

Wei Jiang¹,²*, Zhenwu Luo¹⁴, Sophie Stephenson³, Hong Li⁴, Clara Di Germanio⁵, Philip J Norris⁵, Dietmar Fuchs⁶, Henrik Zetterberg⁷-¹⁰, Magnus Gisslen¹¹,¹², Richard W Price³

¹Department of Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Ave. BSB208D, Charleston, SC, 29425, USA
²Division of Infectious Diseases, Department of Medicine, Medical University of South Carolina, Charleston, SC, 29425, USA
³Department of Neurology, University of California San Francisco, San Francisco General Hospital, San Francisco, CA, 94110, USA
⁴Public Health Sciences, Hollings Cancer Center, Room 400E, Medical University of South Carolina, 86 Jonathan Lucas St, Charleston, SC, 29425, USA
⁵Vitalant Research Institute, San Francisco, CA, 94118, USA
⁶Institut für Biologische Chemie, Biozentrum, Medizinische Universität Innsbruck, Innrain 80 6020 Innsbruck, Austria
⁷Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
⁸Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
⁹Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK
¹⁰UK Dementia Research Institute at UCL, London, UK
¹¹Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 41645, Gothenburg, Sweden
¹²Region Västra Götaland, Sahlgrenska University Hospital, Department of Infectious Diseases, Gothenburg, Sweden

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Summary. In this study, the magnitude of microbial translocation (lipopolysaccharide) associates with neuro-inflammation and blood-brain barrier permeability in HIV without direct penetration into the central nervous system (CNS).
Abstract

HIV infection is associated with increased systemic microbial translocation, neuro-inflammation and occasionally neuronal injury. Whether systemic LPS penetrates into the brain and contributes to neuro-inflammation remain unknown in HIV. Here, we measured plasma and cerebrospinal fluid (CSF) LPS levels along with biomarkers of neuro-inflammation (white blood cell counts and 40 soluble markers) and neurofilament light chain (NfL). Notably, CSF LPS was undetectable in all samples, including three HIV-infected individuals with dementia. Increased plasma LPS, neuro-inflammation, and blood-brain barrier (BBB) dysfunction were found in untreated HIV-infected individuals, but not in healthy or treated HIV-infected individuals. Plasma LPS levels were directly correlated with various markers of inflammation in both plasma and CSF, as well as with degree of BBB permeability but not with CSF NfL in HIV-infected subjects. These results suggest that the magnitude of microbial translocation associates with neuro-inflammation and BBB permeability in HIV without direct penetration into the central nervous system (CNS).

Key words: Cerebrospinal fluid, lipopolysaccharide, HIV-1 infection, neuroinflammation, blood-brain barrier permeability, neuronal injury
Introduction

HIV infection is associated with a “permeable” gut, increased systemic microbial translocation and persistent inflammation even in the presence of viral-suppressive antiretroviral therapy (ART) [1]. HIV-associated neurocognitive disorder (HAND) is more frequently found in patients with abundant systemic and central nervous system (CNS) immune activation, potentially a consequence of microbial translocation [2-4]. While neuroinflammatory responses are primarily a protective mechanism in the brain, persistent inflammation may accelerate neural injury [5]. Indeed, diseases other than HIV infection (e.g., inflammatory bowel diseases) with increased systemic microbial translocation may also associate with accelerated neurocognitive impairment [6, 7]. Furthermore, the plasma levels of markers of monocyte activation (e.g., soluble CD14 (sCD14) and sCD163) through microbial toll-like receptor (TLR)4 agonists associate with neurocognitive impairment in HIV [3, 4]. Monocytes and macrophages are considered to play a key role in HAND, and CD14+CD16+ monocytes are increased in HIV+ patients with dementia and may preferentially migrate through the blood-brain barrier (BBB) [8].

Lipopolysaccharide (LPS) is the major component of the Gram-negative bacterial outer membrane, which binds to TLR4 expressed on the cell surface. Notably, TLR2 and TLR4 are expressed on the human and rat BBB [9], suggesting that ligation of TLR2 and TLR4 may alter the permeability of BBB and induce proinflammatory cytokines to the CNS. Indeed, systemic LPS exposure resulted in increased BBB permeability both in vitro, using human brain microvascular endothelial cells (BMECs), and in vivo in animals [10-13]. Moreover, intraperitoneal injection of LPS resulted in neuroinflammation and behavioral changes [14-16]. However, whether LPS can directly enter and impact the CNS remains controversial.

We undertook this exploratory cross-sectional study measuring plasma and cerebrospinal fluid (CSF) LPS to examine the questions of whether systemically increased LPS is
accompanied by increased CSF LPS and whether plasma LPS, as an indicator of microbial translocation, associates with CNS inflammation and CNS injury in HIV infection.

Methods

Study subjects

This was a cross-sectional exploratory study using paired plasma and CSF samples from 16 healthy individuals, 32 untreated HIV-infected individuals, including 3 who had presented clinically with subacute HIV-associated dementia (HAD), and 27 ART-treated, viral-suppressed HIV-infected individuals. These samples were collected from two academic centers: Sahlgrenska University Hospital, Gothenburg, Sweden and San Francisco General Hospital, University of California San Francisco (UCSF), USA in the context of research protocols approved by the local institutional review boards with informed consent obtained by all participants.

CSF and blood sampling

CSF was obtained according to standard protocols as previously described [17-20]. Subjects also underwent phlebotomy for concurrent blood sampling along with general medical and neurological assessments at the study visit as previously described [21]. CSF was placed immediately on wet ice and subsequently subjected to low-speed centrifugation to remove cells, aliquoted and stored within 2 hours of collection at ≤ -70°C until the time of HIV-1 RNA and biomarker assays. Blood was collected either in EDTA or as serum, aliquoted and stored in parallel with CSF for later batch assays.
Clinical Evaluations

All subjects underwent routine clinical bedside screening for symptoms or signs of CNS opportunistic infections or other conditions that might impact CSF biomarker concentrations; individuals with CNS opportunistic infections or other conditions confounding these analyses were omitted. Designation as ADC/HAD was based on clinicians’ assessment at the time of diagnostic presentation, characteristically with subacute onset and progression of cognitive and motor symptoms and signs. This met American Academy of Neurology criteria in place at the time [22]. Most of these subjects were studied before publication of the more formal Frascati criteria [23] and were diagnosed with AIDS dementia complex (ADC) stages 2-4 [24] but met the functional criteria for the Frascati diagnosis of HAD without the requisite extensive formal neuropsychological assessment.

Background Laboratory Methods

The salient clinical characteristics of these individuals are shown in Table 1. HIV-1 RNA levels were measured in cell-free CSF and plasma using the ultrasensitive Amplicor HIV Monitor assay (versions 1.0 and 1.5; Roche Molecular Diagnostic Systems, Branchburg, NJ), Cobas TaqMan RealTime HIV-1 (version 1 or 2; Hoffmann-La Roche, Basel, Switzerland) or the Abbott RealTime HIV-1 assay (Abbot Laboratories, Abbot Park, IL, USA). All recorded viral loads that were below an LLQ of 20 copies per mL were standardized to a defined ‘floor’ value of 19 copies per mL (log_{10} value of 1.279) for descriptive purposes. Each study visit included assessments by local clinical laboratories using routine methods to measure CSF white blood cell (WBC) count, CSF and blood albumin in order to assess blood-brain barrier integrity [25], and blood CD4+ and CD8+ T lymphocyte counts by flow cytometry. BBB permeability was evaluated by the CSF to serum albumin quotient (Q_{Alb}) [26, 27]. CSF (mg/L) and serum (g/L) albumin levels were analyzed by nephelometry (Behring Nephelometer Analyzer; Behringwerke, Marburg, Germany). CSF neurofilament light chain (NfL) concentration was measured by a sensitive immunoassay using an ELISA kit (NF-
light® ELISA kit, UmanDiagnostics, Umeå, Sweden) in the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital (Gothenburg, Sweden); intra-assay coefficients of variation were below 10% [26]. Since CSF NfL changes with age, CSF NfL levels were age-adjusted to 50 years for comparisons across subjects, and considered normal if below 967 ng/L [28].

Evaluation of LPS

Plasma and CSF LPS levels were measured by endpoint chromogenic limulus amebocyte lysate assays according to the manufacturer’s protocol (Lonza, Basel, Switzerland), described previously [29]. Briefly, CSF samples were from 1:1 to 1:40, and plasma samples were 1:10 diluted with endotoxin-free water and subsequently heated to 85 °C for 15 min to inactivate inhibitory proteins. LPS levels were calculated after subtracting the background values. The limit of LPS detection in both CSF and plasma was 10 pg/mL.

Evaluation of biomarkers of inflammation in CSF and plasma samples

Levels of sCD14 and sCD163 were assessed by ELISA (R&D Systems, Minneapolis, MN). CSF concentrations were measured at the Vitalant Research Institute (San Francisco, CA) while plasma measurements were performed in the Wei Jiang Laboratory. Neopterin was analyzed in Innsbruck, Austria using an ELISA kit (BRAHMS, Berlin, Germany) [30]. Additionally, plasma and CSF levels of 37 inflammatory markers were evaluated using Neuroinflammation Panel 1 kits following the manufacture’s instruction (MESO Scale Discovery, Rockville, MD, USA). The 37 markers are listed in supplemental table 1.
Statistical analysis

The differences in continuous measurements were analyzed by non-parametric Mann-Whitney U tests for two-group comparisons and by the ANOVA and Kruskal-Wallis tests for more than two-group comparisons. Correlations were analyzed by Spearman correlation tests. Age adjusted P values were calculated by ANCOVA using SAS (version 9.3, Cary, NC). All tests were 2-sided, and P values of < 0.05 were considered statistically significant.

Results

*LPS was increased in the plasma of viremic subjects with HIV infection compared to uninfected but was not detected in the CSF of any individual.*

LPS was detected in all 64 plasma samples assayed. By contrast, LPS was not detected in any of the matching CSF samples, including the three individuals with HAD (Figure 1 A and B). Notably, plasma LPS levels were increased in ART-naïve HIV-infected individuals compared to ART-treated patients and HIV-negative controls (Figure 1A). Because age is the key factor associated with CNS function, we calculated P values after adjusting for age by ANCOVA. The differences of plasma LPS between HIV-negative controls and untreated patients (Adjusted P = 0.0002), as well as between the two HIV+ groups (Adjusted P = 0.0004), were still significant after adjusting for age. The difference between ART-treated patients and controls was not significant. Plasma LPS levels of the three HAD patients were not different from the other untreated patients (n = 28), sitting within the middle of the range (Figure 1A).
Similar CSF levels of age-adjusted NfL in HIV-infected individuals and HIV-negative individuals.

To investigate active neuronal injury in HIV-infected individuals, we evaluated age-adjusted NfL levels in the CSF samples. Overall, there was not a difference in age-adjusted NfL levels in CSF among the three study groups, though the three individuals with HAD had notably elevated levels (Figure 1C).

Correlations between plasma LPS levels and the magnitude of BBB permeability as well as CSF WBC counts.

To investigate the link between systemic microbial translocation and neuronal injury, we have analyzed the correlations between plasma LPS levels and CSF NfL levels as well as ratio of albumin levels in the CSF versus serum, a marker of BBB permeability [26, 27]. Notably, the levels blood-brain barrier permeability were increased in HIV+ untreated individuals compared to ART-treated HIV+ or HIV-negative individuals (Table 1). There was no difference of the degree of BBB permeability between ART-treated HIV+ and healthy individuals (Table 1). Plasma LPS levels were correlated with the magnitude of BBB permeability in all individuals (r = 0.24, P = 0.04), all HIV+ subjects (r = 0.42, P = 0.001), and untreated HIV+ subjects (r = 0.44, P = 0.01) but not in ART-treated HIV+ or healthy controls (Figure 1D). Plasma LPS levels were not correlated with CSF NfL levels in any study group (Figure 1D). Consistently, CSF WBC counts were increased in untreated patients compared to treated patients or HIV-negative individuals, but no difference between treated patients and healthy individuals (Table 1). Plasma levels of LPS were correlated with CSF WBC counts in all subjects (r = 0.34, P = 0.003) and all HIV+ subjects (r = 0.45, P = 0.0005) but not in any single HIV study group or healthy controls (Figure 1D).
Correlations between plasma LPS levels and levels of proinflammatory cytokines or chemokines in the blood and CSF samples.

Increased neuroinflammation has been observed in HIV-infected individuals that associated with neurocognitive impairment [2, 31]. We further evaluated 40 soluble markers related to neuroinflammation. Notably, most inflammatory markers were higher in plasma than in CSF, with the exception of IL-6, MCP-1, IL-8, and PIGF (Supplemental Table 1). HIV infection was associated with increases of various inflammatory markers in plasma and CSF; however, both plasma and CSF levels of Flt-1, bFGF, IL-13, IL-15, IL-5, and VEGF-D, and plasma levels of IL-1α, IL-1β, IL-4, MIP-1β, SAA, TARC, Tie-2, TNF-β, and VEGF were similar in HIV+ subjects and controls (Supplemental Table 1). Compared to untreated HIV, ART was associated more significantly with decreased CSF inflammation compared to those in the plasma (Supplemental Table 1). In contrast, compared to untreated HIV, ART was associated with decreases in the plasma sCD14 and sCD163 levels but not those in the CSF (Supplemental Table 1); both plasma sCD14 and sCD163 levels, but not CSF levels, were correlated with plasma LPS in all subjects (Figure 2).

Among the LPS-related inflammatory biomarkers associated with neural injury or HAND [3, 32-35], CSF neopterin levels were increased in untreated HIV+ subjects compared to the other two groups, but no difference was observed between treated patients and healthy controls (Supplemental Table 1). Moreover, plasma sCD14 levels were increased in both treated and untreated HIV+ subjects compared to HIV-negative controls, but CSF sCD14 levels were similar among the three study groups (Supplemental Table 1). There was a correlation between plasma LPS and CSF neopterin (r = 0.47, P < 0.0001) in all subjects, as well as in HIV+ subjects (r = 0.47, P = 0.0002) but not in the healthy control group (Figure 2). There was a correlation between plasma LPS and plasma sCD14 in all subjects only (r = 0.38, P = 0.001, Figure 2). Furthermore, plasma sCD163 levels were increased in untreated HIV+ subjects compared to the other two groups (Supplemental Table 1); CSF sCD163
levels were increased in untreated HIV+ subjects compared to HIV-negative controls, but were similar to those in ART-treated HIV+ subjects (Supplemental Table 1). There was a correlation between plasma LPS and plasma sCD163 in all subjects ($r = 0.41$, $P = 0.0002$) and HIV+ subjects ($r = 0.36$, $P = 0.005$) (Figure 2). However, no correlation was observed between plasma LPS and CSF sCD14 or CSF sCD163 (Figure 2).

Intriguingly, various direct correlations were observed between plasma LPS and levels of inflammatory markers in both plasma and CSF in all subjects (Figure 2A) and all HIV+ subjects (Figure 2B), but few in HIV-negative controls (Figure 2C). The correlations between plasma LPS and CSF levels of neopterin, eotaxin, eotaxin-3, IL-10, IL-1β, IL-2, IL-7, IL-8, MCP-4, MDC, MIP-1β, TARC, TNF-β, and VEGF, were stronger than those between plasma LPS and blood levels of inflammation in both all subjects and all HIV+ subjects (Figure 2A-2B). In contrast, some correlations between plasma LPS and plasma levels of sCD163, Flit-1, IL-6, and MCP-1, were stronger than those between plasma LPS and CSF inflammation in both all subjects and all HIV+ subjects (Figure 2A-2B). These results show the associations between long-term repeated circulating microbial product translocation and inflammation in the blood and CNS in subjects with HIV disease.

**Discussion**

A previous study showed that intravascular injection of LPS into the jugular vein of rats resulted in increased BBB permeability; however, LPS was undetectable in the rodents’ CNS [13]. Another study showed that systemic exposure of LPS resulted in BBB impairment, but LPS did not cross the BBB [12]. In contrast, LPS has been reported to infiltrate to the CNS under certain physiologic conditions in rats [36]. Also, two Alzheimer’s disease studies detected LPS in human primary brain tissues by immunohistochemistry [37, 38]. Moreover, a previous study showed that plasma levels of total bacterial 16S rDNA, a marker of microbial translocation, were correlated with more structural brain abnormalities in HIV patients [39].
This raises the question about cause or consequence of the link between plasma microbial translocation and neuroinflammation and cognitive performance. It remains unknown whether systemic microbial products can enter the CNS in humans. In the current study, we found increased plasma LPS in HIV-infected individuals versus HIV-negative individuals; but LPS was undetectable in the CSF of all subjects including three dementia patients (Figure 1A-1B).

In our model (Figure 3), HIV-associated comprised gut mucosal barrier results in increased plasma LPS, a marker of systemic microbial translocation. The levels of neuroinflammation are low in the healthy individuals due to low plasma levels of LPS as well as an intact BBB barrier. In untreated HIV, high levels of plasma LPS promote a permeable BBB, systemic inflammation, and monocyte activation, which affect neuroinflammation. In viral-suppressed ART-treated HIV, if the mucosal barrier is fully recovered and microbial translocation is limited to similar levels as those of healthy controls, then the neuroinflammation is likely low. However, some HIV+ subjects on virally suppressed ART may exhibit increased plasma levels of LPS, likely with poor CD4+ T cell recovery (immune non-responder) [40, 41]. Increased plasma LPS may contribute to neuroinflammation in the immune non-responders, which deserves further investigation. Thus, mucosal barrier recovery may be a critical factor accounting for plasma LPS-mediated neuroinflammation.

Neuronal injury in HIV-infected individuals may result from persistent neuroinflammation, HIV viral replication, immune activation, oxidative stress, comorbidities and other factors [42]. NfL is a structural component of axons that can be released from damaged neurons to the CSF, thus, increased CSF NfL levels indicate neuronal injury [28]. In the CSF of HIV-infected subjects compared to controls, we found increased neuro-inflammation and BBB permeability; but CSF NfL levels were similar among the three study groups (Figure 1 and Table 1). Previous studies show CSF NfL levels were increased in untreated HIV-infected individuals compared to controls [31, 34]. In the other studies [20, 34, 43], HIV-infected
individuals with HAD had increased levels of CSF NfL, which was consistent with our results (Figure 1C). Nonetheless, our results suggest that increased neuroinflammation is not necessary to result in neural injury and neurodegeneration in HIV. The observation that elevated CSF NfL and HAD is not distinguished by plasma LPS is not consistent with the finding of LPS being associated with CNS injury [44, 45]. However, the cross-sectional nature of the study may limit the evaluation of duration of neuroinflammation and its effects on neuronal damage in HIV.

Systemic LPS exposure resulted in increased BBB permeability in animals in vivo, and in vitro treatment of LPS increased permeability of BMECs [10-13]. Consistently, we found a direct correlation between the plasma LPS level and the degree of BBB permeability in HIV+ subjects (Figure 1D). Furthermore, intraperitoneal injection of LPS in mice resulted in neuroinflammation [14-16]. LPS induced neopterin in human PBMCs and macrophages in vitro [46]. Consistently, we found that plasma LPS levels were correlated with blood and CSF levels of neopterin in HIV. In human studies, correlations were observed between the degree of BBB permeability and CSF neopterin levels and CSF WBC counts, suggesting that CSF neopterin and WBC infiltration may be a consequence of a permeable BBB in HIV [35]. Increased plasma LPS has been reported in HIV+ patients with dementia; plasma sCD14, produced by LPS stimulation in monocytes, is associated with neuro-cognitive impairment in HIV [32, 47, 48]. Plasma but not CSF sCD14 and sCD163, are better markers to associate with HAND [3, 32]. In the current study, significant correlations were observed between plasma LPS and plasma but not CSF levels of sCD14 and sCD163 in all subjects. The disruption of BBB barriers by systemic LPS exposure and TLR-downstream proinflammatory cytokines has been reported in both human and animal studies, but not in HIV [10-13, 49].

In addition to HIV, other diseases (e.g., inflammatory bowel diseases) exhibit increased systemic microbial translocation, accompanied by accelerated neurocognitive disorders [7].
Notably, deletion of CD14+ monocytes attenuated Alzheimer’s disease pathology [50], suggesting monocytes or macrophages play a role in neuro-cognitive impairment. In the current study, we found that plasma LPS was directly correlated with plasma and CSF levels of serial proinflammatory cytokines in all subjects and HIV+ subjects but not in HIV-negative individuals. Some of these cytokines or chemokines can be released from monocytes or macrophages after LPS stimulation. The link between plasma microbial translocation and monocyte activation and migration, as well as their contribution to CNS inflammation and dysfunction, deserves further investigation.

There are several limitations in this exploratory study. These include the relatively small sample size, particularly with respect to the untreated HIV-infected group with a limited range of systemic disease progression and only three individuals with HAD. However, even with this small number, it is clear that LPS does not (or only rarely) enters the CSF in the absence of bacterial infection in the brain or sepsis, so any direct effect of microbial translocation is seemingly confined to its systemic impact. The correlations of plasma LPS with various inflammatory markers in CSF and plasma should likely considered as preliminary and bear examination in a larger and more broadly constructed untreated HIV subjects and treated HIV immune non-responders.
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Conflicts of interest

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pienteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). All other authors have no reported conflicts of interest. This work has not been presented to any meeting.

Corresponding author: Wei Jiang, 173 Ashley Ave. BSB208D. Charleston, SC, 29425.

Email: jianw@musc.edu; Phone number: 843-876-2457.
Figure legends

Figure 1. Increased plasma level of LPS in HIV+ individuals and its direct correlations with the magnitude of BBB permeability, CSF levels of neopterin and WBC counts. (A) Increased plasma level of LPS was found in HIV-infected individuals even after adjusting for age compared to controls. (B) CNS LPS was undetectable in all samples. (C) CSF levels of age-adjusted NfL. Three HIV+ individuals with dementia are shown in filled red circles. (D) Correlations between plasma LPS and albumin ratio in CSF versus serum, CSF age-adjusted NfL level, and CSF WBC counts in HIV-negative controls and HIV-infected individuals.

Figure 2. Correlation coefficient r and P values between plasma LPS levels and inflammation in plasma and CSF samples. Plasma and CSF levels of 40 proinflammatory cytokines and neuropathologic markers were evaluated using MSD neuroinflammation kit or ELISA. The Spearman correlation coefficient (r values in red: positive correlations; in blue: negative correlations) and P values (*) between plasma LPS levels and levels of inflammatory markers in the plasma and CSF in all subjects (A), HIV+ subjects (B), and HIV- subjects (C).

Figure 3. The mechanisms of plasma LPS-mediated neuroinflammation in HIV disease. HIV infection is associated a comprised mucosal barrier (e.g., gut), which results in systemic microbial translocation (e.g., bacterial LPS). There are at least two potential mechanisms accounting for plasma LPS-mediated neuroinflammation in HIV: 1) increased circulating microbial LPS persistently activates monocytes to produce proinflammatory cytokines in the circulation which affect the brain as well; 2) increased circulating microbial LPS persistently activates monocytes to differentiate to M1 macrophages, which migrate to tissue sites such as brain and mediate neuroinflammation.
References


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Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>HIV-negative control</th>
<th>HIV+/untreated</th>
<th>HIV+/ART+/ Sup</th>
<th>P value (HIV+/untreated vs HIV+/ART+ Sup)</th>
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<tr>
<td>Total no. of subjects</td>
<td>16</td>
<td>32</td>
<td>27</td>
<td></td>
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<tr>
<td>Age (yr)</td>
<td>54 (37-62)</td>
<td>49 (35-55)</td>
<td>42 (36-53)</td>
<td>&lt; 0.0001</td>
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<tr>
<td>CD4+ T cell counts</td>
<td>799 (690-914)</td>
<td>255 (98-490)</td>
<td>570 (500-670)</td>
<td></td>
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<tr>
<td>Plasma HIV RNA load</td>
<td>4.9 (4.3-5.5)</td>
<td>1.3 (1.3-1.3)</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>CSF HIV RNA load</td>
<td>3.9 (2.9-5.1)</td>
<td>1.3 (1.3-1.3)</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>CSF WBCs</td>
<td>1.5 (1.0-2.8)</td>
<td>10 (1.5-19)</td>
<td>0 (0-2.0)</td>
<td>&lt; 0.0001</td>
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<tr>
<td>BBB permeability</td>
<td>4.65 (3.9-6.6)</td>
<td>6.3 (4.5-7.3)</td>
<td>4.9 (3.5-5.8)</td>
<td>0.004</td>
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<tr>
<td>QNZP4</td>
<td>-0.06 (-0.42-0.45)</td>
<td>-0.4 (-2.85-0.16)</td>
<td>0.29 (-0.31-0.88)</td>
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CD4+ T cell counts (cells/μL)

HIV RNA (Log_{10} copies/mL)

Data are medians (interquartile ranges)
Figure 1

A. Plasma LPS

B. CSF LPS

C. CSF NFL

D. CSF-blood album ratio

E. CSF WBCs

HIV+ vs. HIV- by ART status:

- All HIV+ & HIV-: r = 0.24, P = 0.04
- HIV+: r = -0.35, P = 0.19
- HIV+: r = -0.42, P = 0.001
- HIV+ ART: r = 0.44, P = 0.03
- HIV+ ART: r = -0.06, P = 0.77

HIV+ vs. HIV- by ART status:

- All HIV+ & HIV-: r = 0.15, P = 0.18
- HIV+: r = -0.02, P = 0.93
- HIV+: r = -0.10, P = 0.44
- HIV+ ART: r = -0.04, P = 0.83
- HIV+ ART: r = -0.05, P = 0.82

HIV+ vs. HIV- by ART status:

- All HIV+ & HIV-: r = 0.24, P = 0.003
- HIV+: r = 0.032, P = 0.99
- HIV+: r = 0.45, P = 0.0005
- HIV+ ART: r = 0.42, P = 0.52
- HIV+ ART: r = -0.08, P = 0.79
Figure 2

Correlation coefficient r & P values

* P < 0.05
** P < 0.01
*** P < 0.001
Figure 3

#1. Systemic inflammation produced by LPS-activated monocytes
- IL-1
- IL-6
- IL-8
- sCD14
- sCD163
- TNF-α

#2. Direct neuroinflammation produced by infiltrated macrophages
- IL-1
- IL-6
- IL-8
- sCD14
- sCD163
- TNF-α

HIV-associated leaky gut

Monocyte

Systemic inflammation

HIV

LPS

TLR4

Human brain

M1 Macrophage

Neuroinflammation