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

Wei Jiang[1,2*#], Zhenwu Luo[1#], Sophie Stephenson[3], Hong Li[4], Clara Di Germanio[5], Philip J Norris[5], Dietmar Fuchs[6], Henrik Zetterberg[7-10], Magnus Gisslen[11,12], Richard W Price[3]

[1]Department of Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Ave. BSB208D, Charleston, SC, 29425, USA

[2]Division of Infectious Diseases, Department of Medicine, Medical University of South Carolina, Charleston, SC, 29425, USA

[3]Department of Neurology, University of California San Francisco, San Francisco General Hospital, San Francisco, CA, 94110, USA

[4]Public Health Sciences, Hollings Cancer Center, Room 400E, Medical University of South Carolina, 86 Jonathan Lucas St, Charleston, SC, 29425, USA

[5]Vitalant Research Institute, San Francisco, CA, 94118, USA

[6]Institut für Biologische Chemie, Biozentrum, Medizinische Universität Innsbruck, Innrain 80 6020 Innsbruck, Austria

[7]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[8]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[9]Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK

[10]UK Dementia Research Institute at UCL, London, UK

[11]Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 41645, Gothenburg, Sweden

[12]Region Västra Götaland, Sahlgrenska University Hospital, Department of Infectious Diseases, Gothenburg, Sweden

© The Author(s) 2020. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

[#]equally contributed

*Corresponding author: Wei Jiang, 173 Ashley Ave. BSB208D, Charleston, SC, USA, 29425; email: jianw@musc.edu.

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).

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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 $\leq$ -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.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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 visits 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-

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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 $\leq$ 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).

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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).

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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].

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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].

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

Acknowledgements

This work was supported by funding from National Institute of Health R01NS094067 (Price) and R01AI1288864 (Jiang), as well as the Swedish government and the county councils (ALF agreement ALFGBG-717531). Dr. Zetterberg is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), and the UK Dementia Research Institute at UCL.

Conflicts of interest

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon 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.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

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.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

References

1. Chung CY, Alden SL, Funderburg NT, Fu P, Levine AD. Progressive proximal-to-distal reduction in expression of the tight junction complex in colonic epithelium of virally-suppressed HIV+ individuals. PLoS Pathog. 2014;10(6):e1004198. doi: 10.1371/journal.ppat.1004198. PubMed PMID: 24968145; PubMed Central PMCID: PMCPMC4072797.
2. Eden A, Marcotte TD, Heaton RK, Nilsson S, Zetterberg H, Fuchs D, et al. Increased Intrathecal Immune Activation in Virally Suppressed HIV-1 Infected Patients with Neurocognitive Impairment. PLoS One. 2016;11(6):e0157160. doi: 10.1371/journal.pone.0157160. PubMed PMID: 27295036; PubMed Central PMCID: PMCPMC4905676.
3. Burdo TH, Weiffenbach A, Woods SP, Letendre S, Ellis RJ, Williams KC. Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection. AIDS. 2013;27(9):1387-95. doi: 10.1097/QAD.0b013e32836010bd. PubMed PMID: 23435298; PubMed Central PMCID: PMCPMC3844286.
4. Ryan LA, Zheng J, Brester M, Bohac D, Hahn F, Anderson J, et al. Plasma levels of soluble CD14 and tumor necrosis factor-alpha type II receptor correlate with cognitive dysfunction during human immunodeficiency virus type 1 infection. J Infect Dis. 2001;184(6):699-706. doi: 10.1086/323036. PubMed PMID: 11517430.
5. Kempuraj D, Thangavel R, Selvakumar GP, Zaheer S, Ahmed ME, Raikwar SP, et al. Brain and Peripheral Atypical Inflammatory Mediators Potentiate Neuroinflammation and Neurodegeneration. Front Cell Neurosci. 2017;11:216. doi: 10.3389/fncel.2017.00216. PubMed PMID: 28790893; PubMed Central PMCID: PMCPMC5522882.
6. Ott C, Scholmerich J. Extraintestinal manifestations and complications in IBD. Nat Rev Gastroenterol Hepatol. 2013;10(10):585-95. Epub 2013/07/10. doi: 10.1038/nrgastro.2013.117. PubMed PMID: 23835489.
7. Moris G. Inflammatory bowel disease: an increased risk factor for neurologic complications. World J Gastroenterol. 2014;20(5):1228-37. Epub 2014/02/28. doi: 10.3748/wjg.v20.i5.1228. PubMed PMID: 24574797; PubMed Central PMCID: PMCPMC3921505.
8. Fischer-Smith T, Croul S, Sverstiuk AE, Capini C, L'Heureux D, Regulier EG, et al. CNS invasion by CD14+/CD16+ peripheral blood-derived monocytes in HIV dementia: perivascular accumulation and reservoir of HIV infection. J Neurovirol. 2001;7(6):528-41. Epub 2001/11/13. doi: 10.1080/135502801753248114. PubMed PMID: 11704885.
9. Nagyoszi P, Wilhelm I, Farkas AE, Fazakas C, Dung NT, Hasko J, et al. Expression and regulation of toll-like receptors in cerebral endothelial cells. Neurochem Int. 2010;57(5):556-64. Epub 2010/07/20. doi: 10.1016/j.neuint.2010.07.002. PubMed PMID: 20637248.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

10. Banks WA, Gray AM, Erickson MA, Salameh TS, Damodarasamy M, Sheibani N, et al. Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. J Neuroinflammation. 2015;12:223. doi: 10.1186/s12974-015-0434-1. PubMed PMID: 26608623; PubMed Central PMCID: PMCPMC4660627.
11. Dohgu S, Fleegal-DeMotta MA, Banks WA. Lipopolysaccharide-enhanced transcellular transport of HIV-1 across the blood-brain barrier is mediated by luminal microvessel IL-6 and GM-CSF. J Neuroinflammation. 2011;8:167. doi: 10.1186/1742-2094-8-167. PubMed PMID: 22129063; PubMed Central PMCID: PMCPMC3260201.
12. Singh AK, Jiang Y. How does peripheral lipopolysaccharide induce gene expression in the brain of rats? Toxicology. 2004;201(1-3):197-207. doi: 10.1016/j.tox.2004.04.015. PubMed PMID: 15297033.
13. Banks WA, Robinson SM. Minimal penetration of lipopolysaccharide across the murine blood-brain barrier. Brain Behav Immun. 2010;24(1):102-9. doi: 10.1016/j.bbi.2009.09.001. PubMed PMID: 19735725; PubMed Central PMCID: PMCPMC2789209.
14. Wendeln AC, Degenhardt K, Kaurani L, Gertig M, Ulas T, Jain G, et al. Innate immune memory in the brain shapes neurological disease hallmarks. Nature. 2018;556(7701):332-8. doi: 10.1038/s41586-018-0023-4. PubMed PMID: 29643512; PubMed Central PMCID: PMCPMC6038912.
15. Lee JW, Lee YK, Yuk DY, Choi DY, Ban SB, Oh KW, et al. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. J Neuroinflammation. 2008;5:37. Epub 2008/09/02. doi: 10.1186/1742-2094-5-37. PubMed PMID: 18759972; PubMed Central PMCID: PMCPMC2556656.
16. Rodrigues FTS, de Souza MRM, Lima CNC, da Silva FER, Costa D, Dos Santos CC, et al. Major depression model induced by repeated and intermittent lipopolysaccharide administration: Long-lasting behavioral, neuroimmune and neuroprogressive alterations. J Psychiatr Res. 2018;107:57-67. doi: 10.1016/j.jpsychires.2018.10.003. PubMed PMID: 30326340.
17. Price RW, Paxinos EE, Grant RM, Drews B, Nilsson A, Hoh R, et al. Cerebrospinal fluid response to structured treatment interruption after virological failure. AIDS. 2001;15(10):1251-9. PubMed PMID: 11426069.
18. Spudich SS, Nilsson AC, Lollo ND, Liegler TJ, Petropoulos CJ, Deeks SG, et al. Cerebrospinal fluid HIV infection and pleocytosis: relation to systemic infection and antiretroviral treatment. BMC Infect Dis. 2005;5:98. doi: 10.1186/1471-2334-5-98. PubMed PMID: 16266436; PubMed Central PMCID: PMCPMC1299327.
19. Andersson LM, Svennerholm B, Hagberg L, Gisslen M. Higher HIV-1 RNA cutoff level required in cerebrospinal fluid than in blood to predict positive HIV-1 isolation. J Med Virol. 2000;62(1):9-13. Epub 2000/08/10. doi: 10.1002/1096-9071(200009)62:1<9::aid-jmv2>3.0.co;2-d. PubMed PMID: 10935982.
20. Gisslen M, Hagberg L, Brew BJ, Cinque P, Price RW, Rosengren L. Elevated cerebrospinal fluid neurofilament light protein concentrations predict the development of AIDS dementia complex. J Infect Dis. 2007;195(12):1774-8. Epub 2007/05/12. doi: 10.1086/518043. PubMed PMID: 17492593.
21. Price RW, Sidtis JJ. Evaluation of the AIDS dementia complex in clinical trials. J Acquir Immune Defic Syndr (1988). 1990;3 Suppl 2:S51-60. Epub 1990/01/01. PubMed PMID: 2231303.
22. Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP). Report from an Ad Hoc Subcommittee of the American Academy of

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

Neurology AIDS Task Force. Neurology. 1991;41(5):617-8. Epub 1991/05/01. PubMed PMID: 2027473.
23. Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, et al. Updated research nosology for HIV-associated neurocognitive disorders. Neurology. 2007;69(18):1789-99. doi: 10.1212/01.WNL.0000287431.88658.8b. PubMed PMID: 17914061; PubMed Central PMCID: PMCPMC4472366.
24. Price RW, Sidtis J, Rosenblum M. The AIDS dementia complex: some current questions. Ann Neurol. 1988;23 Suppl:S27-33. PubMed PMID: 3348598.
25. Spudich S, Gisslen M, Hagberg L, Lee E, Liegler T, Brew B, et al. Central nervous system immune activation characterizes primary human immunodeficiency virus 1 infection even in participants with minimal cerebrospinal fluid viral burden. J Infect Dis. 2011;204(5):753-60. doi: 10.1093/infdis/jir387. PubMed PMID: 21844301; PubMed Central PMCID: PMCPMC3156103.
26. Gaetani L, Hoglund K, Parnetti L, Pujol-Calderon F, Becker B, Eusebi P, et al. A new enzyme-linked immunosorbent assay for neurofilament light in cerebrospinal fluid: analytical validation and clinical evaluation. Alzheimers Res Ther. 2018;10(1):8. doi: 10.1186/s13195-018-0339-1. PubMed PMID: 29370869; PubMed Central PMCID: PMCPMC6389166.
27. Blennow K, Fredman P, Wallin A, Gottfries CG, Karlsson I, Langstrom G, et al. Protein analysis in cerebrospinal fluid. II. Reference values derived from healthy individuals 18-88 years of age. Eur Neurol. 1993;33(2):129-33. doi: 10.1159/000116919. PubMed PMID: 8467819.
28. Yilmaz A, Blennow K, Hagberg L, Nilsson S, Price RW, Schouten J, et al. Neurofilament light chain protein as a marker of neuronal injury: review of its use in HIV-1 infection and reference values for HIV-negative controls. Expert Rev Mol Diagn. 2017;17(8):761-70. Epub 2017/06/10. doi: 10.1080/14737159.2017.1341313. PubMed PMID: 28598205.
29. Luo Z, Li M, Wu Y, Meng Z, Martin L, Zhang L, et al. Systemic translocation of Staphylococcus drives autoantibody production in HIV disease. Microbiome. 2019;7(1):25. Epub 2019/02/16. doi: 10.1186/s40168-019-0646-1. PubMed PMID: 30764863.
30. Yilmaz A, Fuchs D, Price RW, Spudich S, Blennow K, Zetterberg H, et al. Cerebrospinal Fluid Concentrations of the Synaptic Marker Neurogranin in Neuro-HIV and Other Neurological Disorders. Curr HIV/AIDS Rep. 2019;16(1):76-81. doi: 10.1007/s11904-019-00420-1. PubMed PMID: 30649659; PubMed Central PMCID: PMCPMC6420419.
31. Guha D, Mukerji SS, Chettimada S, Misra V, Lorenz DR, Morgello S, et al. Cerebrospinal fluid extracellular vesicles and neurofilament light protein as biomarkers of central nervous system injury in HIV-infected patients on antiretroviral therapy. AIDS. 2019;33(4):615-25. Epub 2018/12/18. doi: 10.1097/QAD.0000000000002121. PubMed PMID: 30557159; PubMed Central PMCID: PMCPMC6399073.
32. Lyons JL, Uno H, Ancuta P, Kamat A, Moore DJ, Singer EJ, et al. Plasma sCD14 is a biomarker associated with impaired neurocognitive test performance in attention and learning domains in HIV infection. J Acquir Immune Defic Syndr. 2011;57(5):371-9. doi: 10.1097/QAI.0b013e3182237e54. PubMed PMID: 21646912; PubMed Central PMCID: PMCPMC3159710.
33. Weaver LK, Pioli PA, Wardwell K, Vogel SN, Guyre PM. Up-regulation of human monocyte CD163 upon activation of cell-surface Toll-like receptors. J Leukoc Biol. 2007;81(3):663-71. doi: 10.1189/jlb.0706428. PubMed PMID: 17164428.
34. Jessen Krut J, Mellberg T, Price RW, Hagberg L, Fuchs D, Rosengren L, et al. Biomarker evidence of axonal injury in neuroasymptomatic HIV-1 patients. PLoS One. 2014;9(2):e88591. Epub 2014/02/14. doi: 10.1371/journal.pone.0088591. PubMed PMID: 24523921; PubMed Central PMCID: PMCPMC3921217.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

35. Anesten B, Yilmaz A, Hagberg L, Zetterberg H, Nilsson S, Brew BJ, et al. Blood-brain barrier integrity, intrathecal immunoactivation, and neuronal injury in HIV. Neurol Neuroimmunol Neuroinflamm. 2016;3(6):e300. doi: 10.1212/NXI.0000000000000300. PubMed PMID: 27868081; PubMed Central PMCID: PMCPMC5104266.
36. Vargas-Caraveo A, Sayd A, Maus SR, Caso JR, Madrigal JLM, Garcia-Bueno B, et al. Lipopolysaccharide enters the rat brain by a lipoprotein-mediated transport mechanism in physiological conditions. Sci Rep. 2017;7(1):13113. Epub 2017/10/17. doi: 10.1038/s41598-017-13302-6. PubMed PMID: 29030613; PubMed Central PMCID: PMCPMC5640642.
37. Zhan X, Stamova B, Jin LW, DeCarli C, Phinney B, Sharp FR. Gram-negative bacterial molecules associate with Alzheimer disease pathology. Neurology. 2016;87(22):2324-32. doi: 10.1212/WNL.0000000000003391. PubMed PMID: 27784770; PubMed Central PMCID: PMCPMC5135029.
38. Zhao Y, Cong L, Jaber V, Lukiw WJ. Microbiome-Derived Lipopolysaccharide Enriched in the Perinuclear Region of Alzheimer's Disease Brain. Front Immunol. 2017;8:1064. doi: 10.3389/fimmu.2017.01064. PubMed PMID: 28928740; PubMed Central PMCID: PMCPMC5591429.
39. Vera JH, Guo Q, Cole JH, Boasso A, Greathead L, Kelleher P, et al. Neuroinflammation in treated HIV-positive individuals: A TSPO PET study. Neurology. 2016;86(15):1425-32. Epub 2016/02/26. doi: 10.1212/WNL.0000000000002485. PubMed PMID: 26911637; PubMed Central PMCID: PMCPMC4831035.
40. Luo ZW LZ, Martin L, Wan Z, Meissner EG, Espinosa E, Wu H, Yu XC, Fu PF, Westerink, MAJ, Kilby JM, Wu J, Huang L, Heath SL, Li ZH, Jiang W. Pathological role of anti-CD4 antibodies in HIV-infected immunologic non-responders under viral suppressive antiretroviral therapy. J Infect Dis. 2017. doi: https://doi.org/10.1093/infdis/jix223.
41. Xu W, Luo Z, Alekseyenko AV, Martin L, Wan Z, Ling B, et al. Distinct systemic microbiome and microbial translocation are associated with plasma level of anti-CD4 autoantibody in HIV infection. Sci Rep. 2018;8(1):12863. doi: 10.1038/s41598-018-31116-y. PubMed PMID: 30150778.
42. Saylor D, Dickens AM, Sacktor N, Haughey N, Slusher B, Pletnikov M, et al. HIV-associated neurocognitive disorder - pathogenesis and prospects for treatment. Nat Rev Neurol. 2016;12(5):309. Epub 2016/04/16. doi: 10.1038/nrneurol.2016.53. PubMed PMID: 27080521; PubMed Central PMCID: PMCPMC5842923.
43. Peterson J, Gisslen M, Zetterberg H, Fuchs D, Shacklett BL, Hagberg L, et al. Cerebrospinal fluid (CSF) neuronal biomarkers across the spectrum of HIV infection: hierarchy of injury and detection. PLoS One. 2014;9(12):e116081. Epub 2014/12/30. doi: 10.1371/journal.pone.0116081. PubMed PMID: 25541953; PubMed Central PMCID: PMCPMC4277428.
44. Vassallo M, Dunais B, Durant J, Carsenti-Dellamonica H, Harvey-Langton A, Cottalorda J, et al. Relevance of lipopolysaccharide levels in HIV-associated neurocognitive impairment: the Neuradapt study. J Neurovirol. 2013;19(4):376-82. Epub 2013/07/13. doi: 10.1007/s13365-013-0181-y. PubMed PMID: 23846287.
45. Monnig MA, Kahler CW, Cioe PA, Monti PM, Mayer KH, Pantalone DW, et al. Markers of Microbial Translocation and Immune Activation Predict Cognitive Processing Speed in Heavy-Drinking Men Living with HIV. Microorganisms. 2017;5(4). Epub 2017/09/22. doi: 10.3390/microorganisms5040064. PubMed PMID: 28934108; PubMed Central PMCID: PMCPMC5748573.
46. Werner-Felmayer G, Werner ER, Fuchs D, Hausen A, Reibnegger G, Wachter H. Tumour necrosis factor-alpha and lipopolysaccharide enhance interferon-induced tryptophan degradation and pteridine synthesis in human cells. Biol Chem Hoppe Seyler.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

1989;370(9):1063-9. Epub 1989/09/01. doi: 10.1515/bchm3.1989.370.2.1063. PubMed PMID: 2482041.
47. Ancuta P, Kamat A, Kunstman KJ, Kim EY, Autissier P, Wurcel A, et al. Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. PLoS One. 2008;3(6):e2516. doi: 10.1371/journal.pone.0002516. PubMed PMID: 18575590; PubMed Central PMCID: PMCPMC2424175.
48. Jespersen S, Pedersen KK, Anesten B, Zetterberg H, Fuchs D, Gisslen M, et al. Soluble CD14 in cerebrospinal fluid is associated with markers of inflammation and axonal damage in untreated HIV-infected patients: a retrospective cross-sectional study. BMC Infect Dis. 2016;16:176. Epub 2016/04/23. doi: 10.1186/s12879-016-1510-6. PubMed PMID: 27103116; PubMed Central PMCID: PMCPMC4839160.
49. Anda T, Yamashita H, Khalid H, Tsutsumi K, Fujita H, Tokunaga Y, et al. Effect of tumor necrosis factor-alpha on the permeability of bovine brain microvessel endothelial cell monolayers. Neurol Res. 1997;19(4):369-76. Epub 1997/08/01. PubMed PMID: 9263215.
50. Reed-Geaghan EG, Reed QW, Cramer PE, Landreth GE. Deletion of CD14 attenuates Alzheimer's disease pathology by influencing the brain's inflammatory milieu. J Neurosci. 2010;30(46):15369-73. Epub 2010/11/19. doi: 10.1523/JNEUROSCI.2637-10.2010. PubMed PMID: 21084593; PubMed Central PMCID: PMCPMC2997622.

Accepted Manuscript

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

Table 1. Clinical characteristics

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

CD4+ T cell counts (cells/µL)

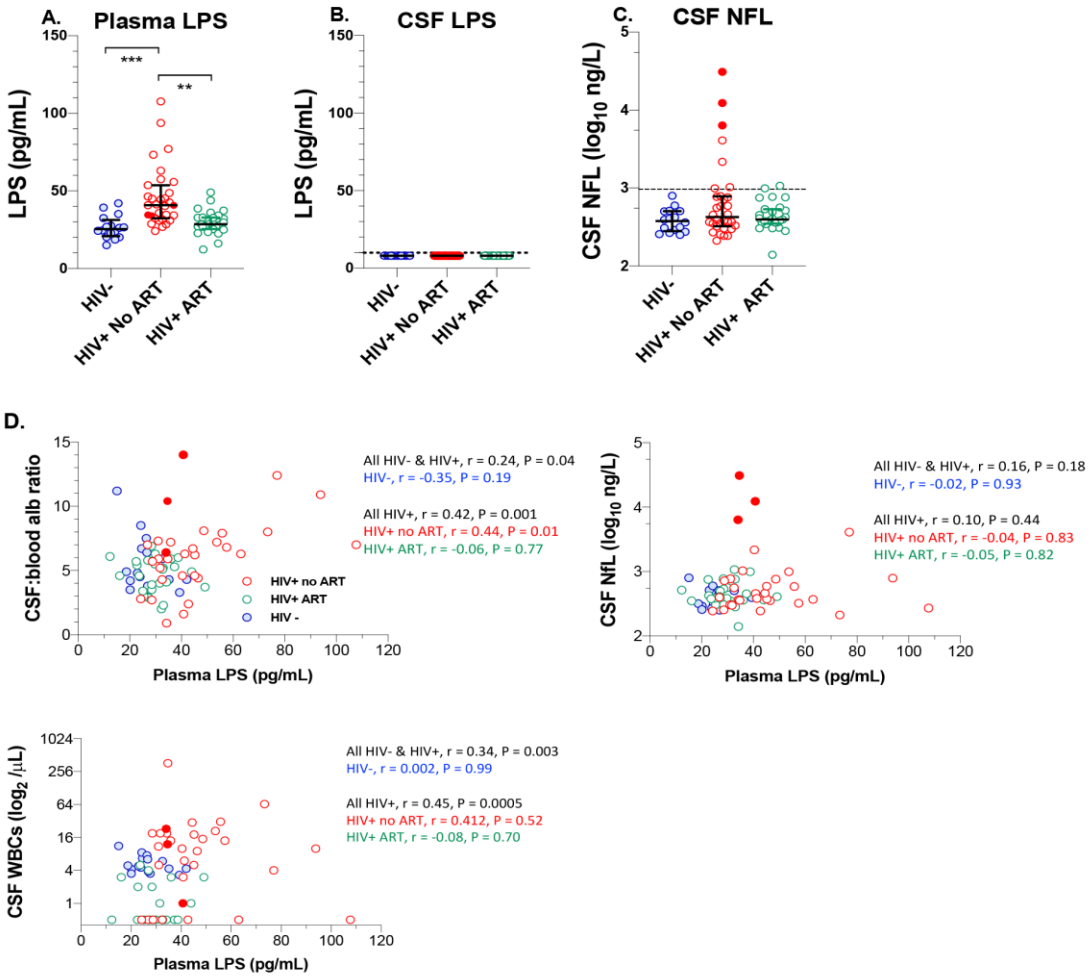
HIV RNA (Log_{10} copies/mL)

Data are medians (interquartile ranges)

Accepted Manuscript

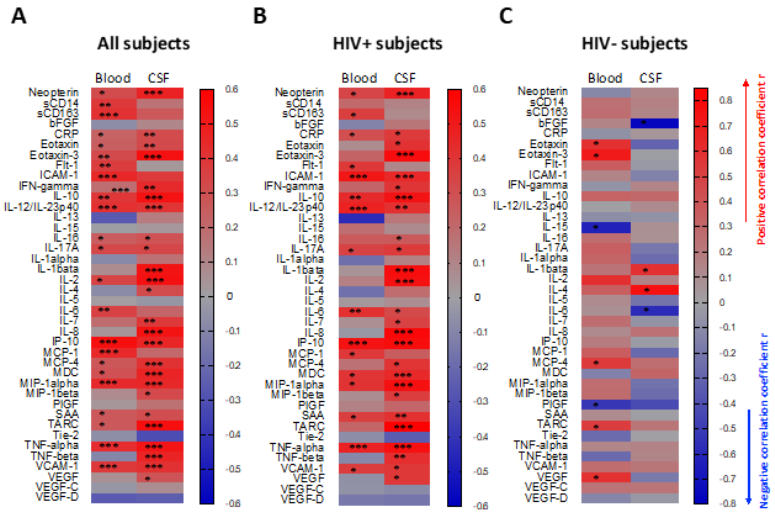
Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

Figure 1



Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

Figure 2

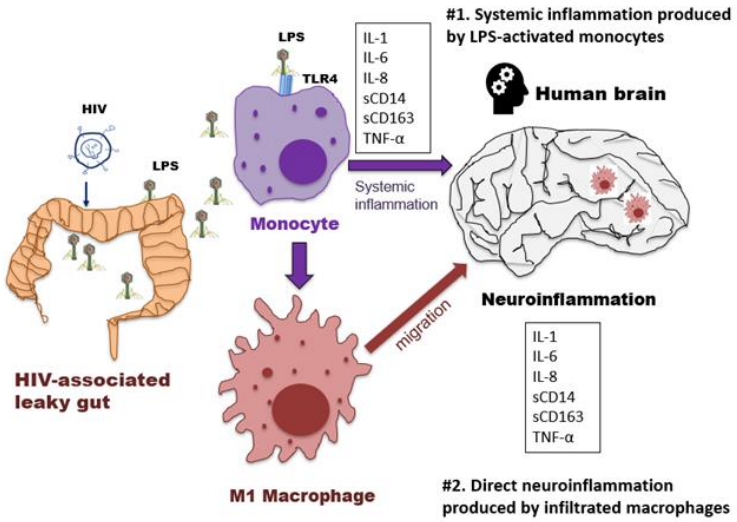


Correlation coefficient r & P values

*, P < 0.05
**, P < 0.001
***, P < 0.0001

Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020

Figure 3



Downloaded from https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa765/6035152 by University College London user on 31 December 2020