Published in final edited form as:

J Neurovirol. 2013 April ; 19(2): 144–149. doi:10.1007/s13365-013-0150-5.

Cytokines in CSF correlate with HIV-associated neurocognitive disorders in the post-HAART era in China

Lin Yuan,

Department of Medicine, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

AIDS Research Beijing Key Laboratory (No: BZ0089), Beijing 100069, China

Luxin Qiao,

Department of Medicine, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

AIDS Research Beijing Key Laboratory (No: BZ0089), Beijing 100069, China

Feili Wei,

Department of Medicine, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

Jiming Yin,

Beijing Institute of Liver Disease, Beijing 100069, China

Lifeng Liu,

Department of Medicine, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

AIDS Research Beijing Key Laboratory (No: BZ0089), Beijing 100069, China

Yunxia Ji,

Department of Medicine, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

Davey Smith,

Division of Infectious Disease, University of California, San Diego, USA

Ning Li, and

Department of Medicine, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

AIDS Research Beijing Key Laboratory (No: BZ0089), Beijing 100069, China

Beijing Institute of Liver Disease, Beijing 100069, China

Dexi Chen

Department of Medicine, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China

AIDS Research Beijing Key Laboratory (No: BZ0089), Beijing 100069, China

Beijing Institute of Liver Disease, Beijing 100069, China

Dexi Chen: dexi0963@yahoo.com

Abstract

In the current era of highly active antiretroviral therapy (HAART), the incidence of HIV dementia has declined, but the prevalence of HIV-associated neurocognitive disorder (HAND) remains high. HIV-induced systemic and localized inflammation is considered to be one of the mechanisms of HAND. Changes in cytokine levels in the cerebrospinal fluid (CSF) during HIV infection might help to identify HAND. To investigate whether the cytokine profile of the CSF during HIV infection could be used as a biomarker of HAND, we compared cytokine levels in the CSF of HIV-infected cases with and without neurocognitive impairment. Cytokine concentrations in the CSF were measured by quantification bioassays (Luminex xMAP). HIV-infected cases with neurocognitive impairment demonstrated higher levels of interleukin (IL)-8, monocyte chemotactic protein (MCP)-1, induced protein (IP)-10, and granulocyte colony-stimulating factor (G-CSF) in the CSF than those without neurocognitive impairment (G-CSF ($p=0.0003$), IL-8 ($p=0.0046$), IP-10 ($p<0.0001$), and MCP-1 ($p<0.0001$)). There was no significant impact of HAART on cytokine levels in the CSF, except for IP-10, which was higher in HAART-treated patients with impaired cognition ($p=0.0182$). Findings from this preliminary study suggest that elevated levels of the cytokines IL-8, MCP-1, G-CSF, and IP-10 in the CSF are associated with neurocognitive impairment in HIV infection, and these cytokines likely represent a biomarker profile for HAND.

Keywords

HIV; Neurocognitive disorder; Cytokines; CSF; Biomarker

Introduction

HIV-associated neurologic disorders (HANDs) are characterized by a spectrum of central nervous system (CNS) complications. Based on disease progression, HAND can range from asymptomatic neurocognitive impairment (ANI) to minor neurocognitive disorder (MND) to the more severe HIV-associated dementia (HAD; Antinori et al. 2007). Highly active antiretroviral therapy (HAART) has decreased the frequency of HAD, but HAND is still common: more than 50 % of HIV-infected persons receiving HAART demonstrate milder HAND syndromes (Heaton et al. 2010), including ANI and MND. Current diagnostic categories of HAND rely on clinical syndromes and emphasize performance on comprehensive neuropsychological testing (Antinori et al. 2007). After more than two decades of investigation, there is still an urgent need for an objective, quantitative, and scalable biomarker profile for HAND (Price et al. 2007; Bandaru et al. 2007; Clifford et al. 2009; McGuire 2009; Nixon et al. 2010). The lack of HAND-specific biomarkers has hindered the diagnosis, risk identification, treatment evaluation, and ongoing management of HAND.

Macrophage and microglial cells are activated during HIV infection and release a number of inflammatory cytokines, which are particularly attractive as potential biomarkers because they likely play an important role in the occurrence and persistence of HAND (Hopkins et al. 1995; Epstein et al. 1993; Aquaro et al. 2005; Williams et al. 2002; Rubin et al. 1999; Persidsky et al. 2000). In this case-control study, we measured and compared cytokine levels in the cerebral spinal fluid (CSF) of HIV patients to determine the relationship between CSF cytokines and HAND.

Methods

Study participants

HIV-infected patients were enrolled in Henan and Yunnan provinces in China, and CSF samples were collected. All CSF samples were centrifuged, and cell-free CSF was aliquoted and immediately frozen at -80°C . The Memorial Sloan-Kettering Scale (MSK) was used to categorize the neurocognitive impairment of each patient. This study was approved by ethics committees at the Capital University of Medical Science in Beijing, and all participants provided informed consent.

Measurement of CSF cytokines

A total of 26 cytokines were measured in the CSF using human cytokine/chemokine MILLIplex kits (Millipore Corp, Billerica, MA, USA): interleukin (IL)-1b, IL-1 receptor agonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage, interferon (IFN)- γ , IFN- α 2, IFN- γ -induced protein (IP)-10, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1 α , MIP-1 β , vascular endothelial growth factor, fibroblast growth factor-2, and TNF- α . The sensitivity of these kits ranged between 0.1 and 10.1 pg/ml for each of the cytokines measured. All samples were assayed concurrently in duplicate. Data were collected using a Bio-Plex Suspension Array Reader (BioRad Laboratories Inc., Hercules, CA, USA). Cytokine concentrations that were lower than the lower limits of detection were reported as undetectable (0).

Statistical analyses

The Mann-Whitney test was used to compare the cytokine concentrations, CD4 $^{+}$ cell counts and HIV RNA levels in different groups. *p* values less than 0.05 were considered statistically significant.

Results

A total of 107 CSF samples was obtained from HIV-1 clade Bor B'-infected individuals (Table 1). The participants included 42 women and 65 men. The risk factors for HIV infection were blood transfusion ($n=18$), intravenous drug use ($n=15$), mother-to-child transmission ($n=2$), paid blood or plasma donation ($n=11$), sexual transmission ($n=52$), and unknown ($n=9$). In total, 51 of 107 patients had CNS complications, including cryptococcal meningitis ($n=30$), tuberculous meningitis ($n=6$), encephalitis ($n=3$), *Toxoplasma* encephalitis ($n=3$), progressive multifocal leukoencephalopathy ($n=2$), cytomegalovirus

radiculitis ($n=1$), and others ($n=6$). Nearly half of the HIV-infected patients (43 %) were receiving HAART at the time of evaluation. All were on multidrug combination ART regimens, which consisted of at least two NRTIs (e.g., AZT, D4T, 3TC, DDI, and TDF) plus an NNRTI (NVP or EFV) or a PI (LPV). None were on mono or dual therapy. Based on MSK classification, patients were classified into HIV infected with normal cognition (HIV-NC; $n=43$, MSK=0) and impaired cognition groups (HIV-CI; $n=64$); the HIV-CI group consisted of MSK=0.5 ($n=26$), MSK=1 ($n=15$), MSK=2 ($n=14$), and MSK=3 ($n=9$).

Overall, 26 cytokines were measured in CSF samples. The concentrations of G-CSF ($p=0.0003$), IL-8 ($p=0.0046$), IP-10 ($p<0.0001$), and MCP-1 ($p<0.0001$) in the CSF from the HIV-CI group were significantly higher than those from the HIV-NC group (Fig. 1a), whereas the rest of the tested cytokines were either not detectable or not significantly different (data not shown). In contrast, there was no significant difference in the HIV RNA level in the CSF between the two groups (Fig. 1b).

To account for the potential effect of HAART, we divided the HIV-CI and HIV-NC groups into HAART-treated and untreated subgroups. The cytokine levels showed no difference, except for IP-10, which was higher in HAART-treated HIV-CI patients ($p=0.0182$; Fig. 2a). HIV-infected patients benefitted from HAART therapy, as the HIV viral burden in the plasma and CSF was significantly lower than in untreated patients (Fig. 2b). The impact of HAART on the CD4⁺ cell count was not obvious in our study (Fig. 2c).

Discussion

Chronic immune activation in the CNS during HIV infection is a major contributor to HAND, and inflammatory cytokines likely play an important role, as their levels correlate with HIV infection progression (Letendre et al. 2011; Bebell et al. 2008; Roberts et al. 2010). In our study, the concentrations of IL-8, IP-10, MCP-1, and G-CSF in the CSF of HIV patients with neurocognitive impairment were higher than those in normal cognition patients. IL-8 is the endogenous ligand of CXCR1 and CXCR2, which are mainly produced by monocytes and macrophages, and it is present in increased amounts during brain injury and neuroinflammation. Furthermore, HIV-1 gp120 can induce IL-8 expression in human brain microvascular endothelial cells through a STAT1-dependent pathway and in astrocytes at both the RNA and protein levels (Yang et al. 2009; Shah et al. 2010). MCP-1, also known as CC chemokine ligand 2, is capable of attracting and activating monocytes and macrophages. Although MCP-1 provides partial protection at the early stage of viral infection, once infection has been established, MCP-1 serves primarily as a mediator of inflammation and leukocyte recruitment (Eugenin et al. 2006; Gonzalez et al. 2002). Additionally, HIV-1 Nef upregulates MCP-1 expression in astrocytes in a myristoylation- and calmodulin-dependent manner (Lehmann et al. 2006). Interferon- γ -inducible protein 10 (IP-10/CXCL10) is expressed by astrocytes and microglia in the brain, acts specifically on activated T cells and macrophages and attracts T lymphocytes into the CSF. Similar to IL-8, IP-10 can also be induced by gp120 in addition to HIV-1 Nef and Tat, and IP-10 cannot only inhibit viral replication by competing with HIV-1 for binding to its coreceptor but also stimulate HIV-1 replication by increasing the accumulation of products of reverse transcription in both infected T cells and macrophages (Lane et al. 2003). IP-10 has been

associated with SHIV encephalitis in macaques (Sui et al. 2003). In short, elevated concentrations of these cytokines in the CSF lead to the migration of peripheral macrophages through the blood–brain barrier, the recruitment of inflammatory cells and the activation of the immune response in the brain, which initially provides neuron protection but is usually followed by an inflammatory response in the CNS that is neurotoxic. This study supports the hypothesis that local inflammation is related to neurocognitive impairment during HIV infection. The cytokines involved may offer a viable biomarker profile for HAND.

Individual patients may exhibit dramatic improvement in cognitive function after taking HAART, but this is not the case for most patients with HAND. Recent studies indicate that HAART has only modest benefits in reducing HIV-associated cognitive impairment (Robertson et al. 2007; Al-Khindi et al. 2011). Although antiviral drugs with high CNS penetration scores, such as zidovudine and nevirapine, are commonly used in the Chinese free antiviral strategy, in our study, neurocognitive impairment was not associated with HIV viral load in the CSF. The levels of IL-8, MCP-1, and G-CSF in the CSF were not significantly different between HAART-treated and untreated patients, which might suggest that HAART treatment has limited effect on local inflammation in the CNS during HIV infection. The minor impact of HAART on these cytokine concentrations in the CSF might enhance their usefulness as HAND biomarkers in the post-HAART era.

In our study, 47.7 % of subjects had CNS comorbidities (Table 1). We did not find that the cytokine level in the CSF from HIV-CI patients was different from HIV-NC patients. Due to the limitation of self-reporting CNS comorbidities that lack detailed disease information, such as organism burden, clinical investigation, or therapy, the impact of CNS comorbidity on cytokine levels in the CSF requires further validation.

In summary, HAND remains a significant problem and is highly prevalent, even in the current era of HAART use. This study identified that elevations in the IL-8, MCP-1, IP-10 and G-CSF levels in the CSF during HIV infection are associated with neurocognitive impairment, indicating a useful biomarker profile for diagnosing HAND and guiding clinical therapeutic interventions.

Acknowledgments

This work was supported by grants from the International Cooperation and Exchange of the National Natural Science Foundation of China (30910103915), the Beijing Municipal Natural Science Foundation (7092045, 7101005), the Chinese National Twelfth Five Year Key Scientific and Technological Project (2012ZX10001-002, 2012ZX10001-003, and 2012ZX10001-004), and the Beijing Science and Technology Program (Z111107058811071).

References

- Al-Khindi T, Zakzanis KK, van Gorp WG. Does antiretroviral therapy improve HIV-associated cognitive impairment? A quantitative review of the literature. *J Int Neuropsychol Soc.* 2011; 17:956–69.10.1017/S1355617711000968 [PubMed: 21813033]
- Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, et al. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology.* 2007; 69:1789–99. [PubMed: 17914061]

- Aquaro S, Ronga L, Pollicita M, Antinori A, Ranazzi A, Perno CF. Human immunodeficiency virus infection and acquired immunodeficiency syndrome dementia complex: role of cells of monocyte-macrophage lineage. *J Neurovirol*. 2005; 11:58–66.10.1080/13550280500513416 [PubMed: 16540457]
- Bandaru VV, McArthur JC, Sacktor N, Cutler RG, Knapp EL, Mattson MP, et al. Associative and predictive biomarkers of dementia in HIV-1-infected patients. *Neurology*. 2007; 68:1481–7. [PubMed: 17470750]
- Bebell LM, Passmore JA, Williamson C, Mlisana K, Iriogbe I, Logger-enberg F, et al. Relationship between levels of inflammatory cytokines in the genital tract and CD4+ cell counts in women with acute HIV-1 infection. *J Infect Dis*. 2008; 198:710–4.10.1086/590503 [PubMed: 18643751]
- Clifford DB, Fagan AM, Holtzman DM, Morris JC, Teshome M, Shah AR, et al. CSF biomarkers of Alzheimer disease in HIV-associated neurologic disease. *Neurology*. 2009; 73:1982–7. [PubMed: 19907013]
- Epstein LG, Gendelman HE. Human immunodeficiency virus type-1 infection of the nervous system: pathogenetic mechanisms. *Ann Neurol*. 1993; 33:429–36. [PubMed: 8498818]
- Eugenin EA, Osiecki K, Lopez L, Goldstein H, Calderon TM, Berman JW. CCL2/monocyte chemoattractant protein-1 mediates enhanced transmigration of human immunodeficiency virus (HIV)-infected leukocytes across the blood–brain barrier: a potential mechanism of HIV-CNS invasion and NeuroAIDS. *J Neurosci*. 2006; 26:1098–106.10.1523/JNEUROSCI.3863-05.2006 [PubMed: 16436595]
- Gonzalez E, Rovin BH, Sen L, Cooke G, Dhanda R, Mummidi S. HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels. *Proc Natl Acad Sci U S A*. 2002; 99:13795–800. [PubMed: 12374865]
- Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*. 2010; 75:2087–96. [PubMed: 21135382]
- Hopkins SJ. Cytokines and the nervous system. I: Expression and recognition. *Trends Neurosci*. 1995; 18:83–8. [PubMed: 7537419]
- Lane BR, King SR, Bock PJ, Strieter RM, Coffey MJ, Markovitz DM. The C-X-C chemokine IP-10 stimulates HIV-1 replication. *Virology*. 2003; 307:122–34.10.1016/S0042-6822(02)00045-4 [PubMed: 12667820]
- Lehmann MH, Masanetz S, Kramer S, Erfle V. HIV-1 Nef upregulates CCL2/MCP-1 expression in astrocytes in a myristoylation-and calmodulin-dependent manner. *J Cell Sci*. 2006; 119:4520–30.10.1242/jcs.03231 [PubMed: 17046994]
- Letendre SL, Zheng JC, Kaul M, Yiannoutsos CT, Ellis RJ, Taylor MJ, et al. Chemokines in cerebrospinal fluid correlate with cerebral metabolite patterns in HIV-infected individuals. *J Neurovirol*. 2011; 17:63–9.10.1007/s13365-010-0013-2 [PubMed: 21246320]
- McGuire D. CSF biomarkers in HIV dementia: through a glass darkly. *Neurology*. 2009; 73:1942–4. [PubMed: 19907014]
- Nixon DE, Landay AL. Biomarkers of immune dysfunction in HIV. *Curr Opin HIV AIDS*. 2010; 5(6): 498–503.10.1097/COH.0b013e32833ed6f4 [PubMed: 20978393]
- Persidsky Y, Zheng J, Miller D, Gendelman HE. Mononuclear phagocytes mediate blood–brain barrier compromise and neuronal injury during HIV-1-associated dementia. *J Leukoc Biol*. 2000; 68:413–22. [PubMed: 10985259]
- Price RW, Epstein LG, Becker JT, Cinque P, Gisslen M, Pulliam L, et al. Biomarkers of HIV-1 CNS infection and injury. *Neurology*. 2007; 69:1781–8. [PubMed: 17967994]
- Roberts L, Passmore JA, Williamson C, Little F, Bebell LM, Mlisana K, et al. Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. *AIDS*. 2010; 24:819–31.10.1097/QAD.0b013e3283367836 [PubMed: 20224308]
- Robertson KR, Smurzynski M, Parsons TD, Wu K, Bosch RJ, Wu J, et al. The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS*. 2007; 21:1915–21.10.1097/QAD.0b013e32828e4e27 [PubMed: 17721099]
- Rubin L, Staddon J. The cell biology of the blood–brain barrier. *Ann Rev NeuroSci*. 1999; 22:11–27.10.1146/annurev.neuro.22.1.11 [PubMed: 10202530]

- Shah A, Kumar A. HIV-1 gp120-mediated increases in IL-8 production in astrocytes are mediated through the NF- κ B pathway and can be silenced by gp120-specific siRNA. *J Neuroinflammation*. 2010; 7:96–101. [PubMed: 21190575]
- Sui Y, Potula R, Pinson D, Adany I, Li Z, Day J, et al. Microarray analysis of cytokine and chemokine genes in the brains of macaques with SHIV-encephalitis. *J Med Primatol*. 2003; 32:229–39.10.1034/j.1600-0684.2003.00030.x [PubMed: 14498983]
- Williams K, Hickey WF. Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. *Annu Rev Neurosci*. 2002; 25:537–62.10.1146/annurev.neuro.25.112701.142822 [PubMed: 12052920]
- Yang B, Akhter S, Chaudhuri A, Kanmogne GD. HIV-1 gp120 induces cytokine expression, leukocyte adhesion, and transmigration across the blood–brain barrier: modulatory effects of STAT1 signaling. *Microvasc Res*. 2009; 77:212–9.10.1016/j.mvr.2008.11.003 [PubMed: 19103208]

Fig. 1.

Comparison of G-CSF, IL-8, IP-10, and MCP-1 concentrations in the CSF in samples from 64 HIV-infected subjects with impaired cognition (HIV-CI) and 43 HIV-infected subjects with normal cognition (HIV-NC). Values below the lower limit of detection of the assay are reported as zero (a). HIV RNA levels in the CSF were also compared (b). The levels of G-CSF, IL-8, IP-10, and MCP-1 in the CSF in the HIV-CI group were significantly higher than in the HIV-NC group, whereas no difference was found in the HIV RNA levels in the CSF. Differences were analyzed by the Mann–Whitney *U* test. *p* values of <0.05 were considered significant. *Dots* cytokine or RNA level in the CSF for each study subject, *horizontal lines* median values for each group

Fig. 2.

The concentrations of G-CSF, IL-8, IP-10, and MCP-1 in the CSF were compared between HAART-treated and untreated patients in the HIV-CI group and HIV-NC group. There was no significant difference, except for IP-10, which was higher in HAART-treated patients than in untreated patients within the HIV-CI group ($p=0.0182$; a). HIV RNA levels in the CSF and plasma were lower in HAART-treated patients than in untreated patients (b), whereas CD4⁺ cells showed no difference between the two groups (c). The differences between groups were calculated using the Mann–Whitney U test. P values of <0.05 were

considered significant. Dots cytokine level, viral load or CD4+ cell count for each study subject; *horizontal lines* median values for each group

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Demographic and disease characteristics of subjects

Characteristics	HIV-CI		HIV-NC	
	Untreated	HAART treated	Untreated	HAART treated
No. of subjects	39	25	22	21
Age in years	39±13	39±9	40±9	36±10
Gender [<i>n</i> (%)]				
Male	31 (79.4)	13 (52.0)	13 (59.1)	15 (71.4)
Female	8 (20.5)	12 (48.0)	9 (40.9)	6 (28.6)
HIV RNA (log10 copies/ml)				
CSF	3.57±1.25	2.93±1.33	3.84±1.13	2.96±1.22
Plasma	4.33±1.09	3.39±1.56	4.79±0.84	3.43±1.08
CD4+ cell count (cell/μl)	60±53	109±138	71 ±70	139±173
CNS complications [<i>n</i> (%)]	15 (38.5)	16 (64.0)	8 (36.4)	12 (57.1)