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Cerebrospinal Fluid HIV-1 Viral Load during Treatment of Cryptococcal Meningitis

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Human immunodeficiency virus (HIV-1) can be detected in the cerebrospinal fluid (CSF) throughout the course of HIV infection, although at lower concentrations than in blood.¹ Opportunistic meningeal infections, such as *Cryptococcus neoformans* and *Mycobacterium tuberculosis* can enhance HIV replication in vitro,²⁻⁴ and during such infections, HIV-1 viral load (VL) in CSF is found to be raised,⁵ either as a result of this direct enhancement of replication, and/or increased HIV-1 replication due to a pro-inflammatory immune response and the influx of leucocytes.⁶ Thus, treatment of the opportunistic infection might lead to a more rapid decline of the HIV-1 VL in the compartment of infection than systemically.

To examine the changes in CSF and plasma HIV-1 VL during treatment of cryptococcal meningitis, we measured the levels of HIV-1 RNA in CSF and plasma before and after 2 weeks of treatment of cryptococcal meningitis in 63 Thai patients enrolled in a trial of amphotericin B-based therapy.⁷ Baseline cryptococcal organism load was determined by quantitative CSF cultures, as were CSF cell count and differential and CSF and plasma cytokines (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, G-CSF, GM-CSF, MCP-1, MIP-1 β , IFN- γ , and TNF- α)⁸ to investigate any association of CSF immune parameters with CSF HIV-1 RNA concentrations.

CSF was available from 57 patients at baseline and 47 at day 14. Plasma was available from 62 patients at baseline and 49 at day 14. Paired CSF/plasma samples were available of 57 patients at baseline, and 46 at day 14. CSF and plasma samples were centrifuged at 3000 rpm for 10 minutes at 4°C and supernatants were stored at –80°C. The mean time from lumbar puncture to storage at –80°C was less than 3 hours. After collecting all samples, HIV RNA quantification in the cell-free CSF and plasma was performed with the Roche

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Amplicor Monitor Test (version 1.5, Roche Diagnostic Systems, Hoffman-La Roche, Basel, Switzerland). We compared median $\log_{10}$ HIV-1 RNA concentrations using Wilcoxon matched pairs signed-rank test and the Mann Whitney U test. Correlation between variables was tested using Spearman's rank correlation.

Median $\log_{10}$ HIV-1 VL was significantly lower in CSF compared to plasma at baseline and at day 14 ($p < 0.001$ for both comparisons). There was a significant decrease in HIV-1 VL in both CSF and plasma during the first two weeks of treatment of cryptococcal meningitis (Table). However, the CSF/Plasma HIV-1 VL ratio did not change over time. Baseline CSF HIV-1 VL correlated with CSF lymphocyte count ($r=0.5$, $P=0.0002$). At baseline, there was a positive correlation between CSF IL-10 and CSF HIV-1 VL ($r=0.6$, $P<0.0001$) and a weak correlation between CSF IL-6 and CSF HIV-1 VL ($r=0.3$, $P=0.03$). CSF MCP-1 was negatively correlated with CSF HIV-1 VL ($r=-0.5$, $P=0.0005$). No other CSF or plasma cytokines were associated with VL.

The patients in this study were profoundly immune-depressed with a median CD4 count of $9 \times 10^6/L$ (IQR $6-17 \times 10^6/L$) and thus had a high probability of having systemic cryptococcosis,⁹ which might have caused enhanced HIV-1 replication in both plasma and CSF. Treatment with systemic anti-fungal therapy could reduce HIV-1 VL locally and systemically through a reduction in cryptococcal antigens, known to enhance HIV-1 replication, or alternatively, or in addition, a reduction in the inflammatory response in both systemic and localized compartments.

Our results suggest this latter mechanism may play a role. From prior work we know that the CSF inflammatory response is markedly diminished by 2 weeks into antifungal therapy.⁸ In addition, we did not find a positive relation between the cryptococcal colony forming units (and cryptococcal antigen) and the HIV viral load in CSF. Instead we found a weak inverse correlation between baseline cryptococcal colony forming unit count and HIV-1 RNA concentrations ($r=-0.3$, $P=0.02$). However, in prior work we have shown that baseline cryptococcal organism load is negatively correlated with CSF pro-inflammatory cytokine concentrations.⁸ Thus it is possible a brisk pro-inflammatory cytokine response controls infection more effectively and so is associated with a lower baseline cryptococcal organism load, but also increases HIV-1 VL through enhanced lymphocyte influx into the CSF and enhanced HIV-1 replication. We did find a positive correlation between the number of lymphocytes and HIV-1 VL in the CSF, as was found before by Morris,⁵ although we cannot determine the relative importance of HIV-1 replication in activated lymphocytes in the CSF, as opposed transportation of HIV-1 into the CSF by these lymphocytes. We found a weak correlation between IL-6 and HIV-1 VL in the CSF, whereas there was a strong correlation between HIV-1 VL and IL-10 concentrations. The role of IL-10 in HIV-1 replication seems to be bifunctional.¹⁰ In vitro both reduction and enhancement of HIV-1 replication has been shown in macrophages. In T-lymphocytes, however, IL-10 failed to inhibit HIV-1 replication. The strong correlation between HIV-1 RNA and IL-10 in the CSF in our study suggests an enhancing role of IL-10 on the replication of HIV-1 in the lymphocytes in the CSF.

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REFERENCES

1. Gisslen M, Fuchs D, Svennerholm B, et al. Cerebrospinal fluid viral load, intrathecal immunoactivation, and cerebrospinal fluid monocytic cell count in HIV-1 infection. *J Acquir Immune Defic Syndr*. 1999; 21:271–276. [PubMed: 10428104]
2. Lederman MM, Georges DL, Kusner DJ, et al. Mycobacterium-Tuberculosis and Its Purified Protein Derivative Activate Expression of the Human-Immunodeficiency-Virus. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1994; 7:727–733.
3. Pettoellomantovani M, Casadevall A, Kollmann TR, et al. Enhancement of Hiv-1 Infection by the Capsular Polysaccharide of Cryptococcus-Neoformans. *Lancet*. 1992; 339:21–23. [PubMed: 1370335]
4. Harrison TS, Nong SH, Levitz SM. Induction of human immunodeficiency virus type 1 expression in monocytic cells by Cryptococcus neoformans and Candida albicans. *J Infect Dis*. 1997; 176:485–491. [PubMed: 9237716]
5. Morris L, Silber E, Sonnenberg P, et al. High human immunodeficiency virus type 1 RNA load in the cerebrospinal fluid from patients with lymphocytic meningitis. *J Infect Dis*. 1998; 177:473–476. [PubMed: 9466541]
6. Lawn SD, Butera ST, Folks TM. Contribution of immune activation to the pathogenesis and transmission of human immunodeficiency virus type 1 infection. *Clin Microbiol Rev*. 2001; 14:753–777. [PubMed: 11585784]
7. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. *Lancet*. 2004; 363:1764–1767. [PubMed: 15172774]
8. Siddiqui AA, Brouwer AE, Wuthiekanun V, et al. IFN-gamma at the site of infection determines rate of clearance of infection in cryptococcal meningitis. *J Immunol*. 2005; 174:1746–1750. [PubMed: 15661940]
9. Casadevall, A.; Perfect, JR. *Cryptococcus neoformans*. American Society for Microbiology; Washington DC: 1998. p. 407-456.
10. Poli G. Cytokines and the human immunodeficiency virus: from bench to bedside. *Eur J Clin Invest*. 1999; 29:723–732. [PubMed: 10457158]

Table

Baseline and Day 14 CSF and Plasma HIV-1 RNA concentration

There was a significant decrease of HIV-1 RNA concentrations in CSF and plasma during the first two weeks of treatment of cryptococcal meningitis ($P= < 0.01$), whilst the ratio of CSF:plasma HIV-1 RNA remained unchanged.

	Baseline			Day 14			P*
	N	Median	IQR	N	Median	IQR	
CSF log ₁₀ HIV-1 RNA (log ₁₀ copies/ml)	57	4.94	4.38 5.34	47	4.55	4.06 5.06	0.01 ^a
Plasma log ₁₀ HIV-1 RNA (log ₁₀ copies/ml)	62	5.30	5.02 5.52	49	5.04	4.87 5.30	0.0003 ^b
CSF:P log ₁₀ HIV-1 RNA	57	0.927	0.856 1.04	46	0.922	0.845 0.999	0.47 ^c

* Wilcoxon matched pairs signed-rank test;

^a n=43;

^b n=48;

^c n=42