CD14+ PBMC Secrete Cytokines Linked to HIV-Associated Neurocognitive Disorders

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Abstract

Background. HIV-associated neurocognitive disorders (HAND) persist despite the availability of combined antiretroviral therapy (cART) and believed to be at least partially the consequence of mechanisms associated with monocytes. In addition to transporting HIV into the brain, monocytes secrete pro-inflammatory cytokines that lead to neuronal damage. In this study, we analyzed cytokines that were secreted from CD14-selected peripheral blood mononuclear cells (PBMC) from HIV-infected individuals with HAND and normal cognition (NC) at baseline (cART naïve) and after one year on cART to determine which cytokines were associated with HAND. **Methods.** The study population consisted of 61 HIV-infected Thais who were enrolled in SEARCH011 (NCT00782808); 28 diagnosed with HAND and 33 with NC at entry. PBMC were collected at baseline and 12 months post cART initiation. CD14+ PBMC were separated by magnetic beads and cultured overnight (median 91.9% purity by flow cytometry). Cytokine secretions were measured from the supernatants captured after 24 hour culture and using a custom 10-plex Milliplex MAP kit detecting fractalkine, IFN-g, IL-2, IL-4, IL-6, IL-8, IL-10, IP-10, MCP-1, and TNF-α. HIV DNA copies were also analyzed from the CD14+ PBMC using a real-time qPCR. Non-parametric Spearman correlation and Wilcoxon rank-sum test were conducted. **Results.** Of the cytokines analyzed, only IL-8 and MCP-1 levels were significantly higher in those with HAND in comparison to NC at baseline ($p<0.003$). HIV DNA levels were directly correlated to IL-8 ($r=0.33$; $p=0.01$) and MCP-1 ($r=0.39$; $p=0.003$) at baseline but not after one year. **Conclusions.** This study demonstrated that individuals with HAND experience continued inflammation and the type of cytokine supports monocyte involvement consistent with their likely role as viral reservoirs that continue to persist despite cART. High levels of IL-8 and MCP-1 continued to be secreted by CD14+ PBMC in individuals with HAND despite initiation of cART. We hypothesize that secretion of these cytokines may play an important continued role in promoting the continued transmigration of monocytes into the brain that leads to the persistence of HAND despite cART.

Background

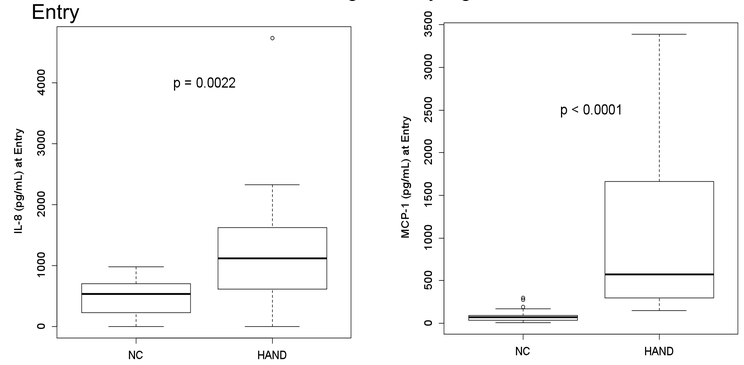
- HIV-associated neurocognitive disorders (HAND) continue to affect HIV-infected individuals even with effective combined antiretroviral therapy (cART)
- It is hypothesized that HIV can be transported into the brain through HIV-infected monocytes that are able to cross the blood brain barrier[1]
- Once in the brain, HIV causes inflammation and neuronal damage that ultimately results in the development of HAND[2]
- Monocytes and macrophages can also form viral reservoirs that contribute to the persistance of HAND because they provide a constant source of inflammation through cytokine production[3]
- Viral reservoirs can be identified through the measurement of HIV DNA
- In this study, we measured cytokines in CD14+ enriched PBMC cultures from individuals with HAND and normal cognition (NC) to characterize their phenotype
- Variable and discordant cytokine levels have been shown from plasma; therefore, CD14+ enriched PBMC cultures were used

Methods

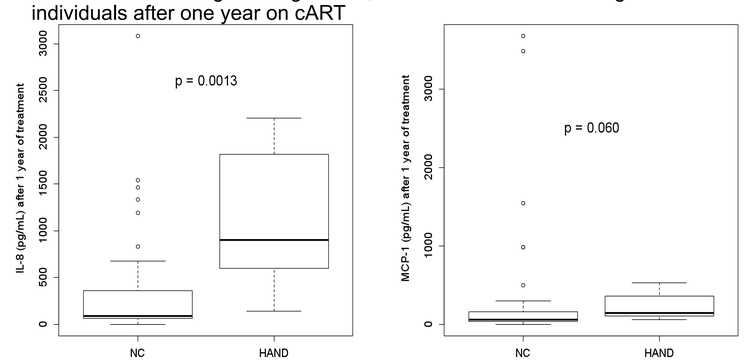
- The study subjects consisted of 61 HIV-infected Thais enrolled in SEARCH011 (NCT00782808) of which 28 were diagnosed with HAND and 33 with NC at entry
- Peripheral blood mononuclear cells (PBMC) were collected at baseline (entry) and after one year on cART (12 months)
- PBMC were enriched for CD14+ cells (monocytes) using a magnetic bead positive selection kit (Miltenyi Biotec) with a median 91.9% purity determined by flow cytometry
- CD14+ enriched PBMC were cultured overnight and the following inflammatory cytokines: Fractalkine, IFN-g, IL-2, IL-4, IL-6, IL-8, IL-10, IP-10, MCP-1, and TNF-α were analyzed in the tissue culture supernatant using a custom 10-plex Milliplex MAP kit (EMD Millipore) on a Luminex 100 system
- HIV DNA levels were measured in the CD14+ PBMC through the amplification of the *gag* and *b-glo* genes[4]
- Statistics were completed with SAS version 9.3 using Wilcoxon rank-sums and Kruskal-Wallis tests. Correlations were evaluated through nonparametric Spearman Correlation with a two-sided $p<0.05$ regarded as significant

Results

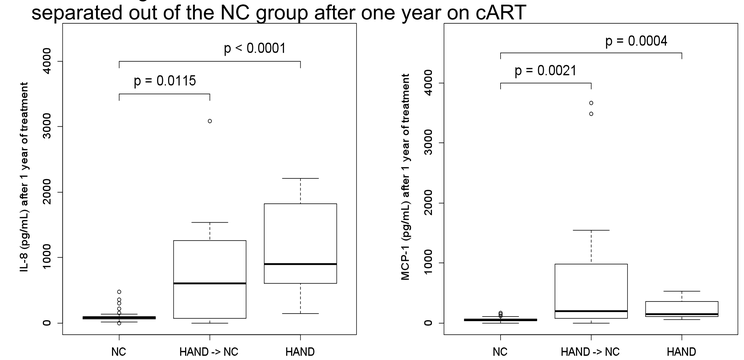
- IL-8 and MCP-1 secretions were significantly higher in HAND individuals at Entry



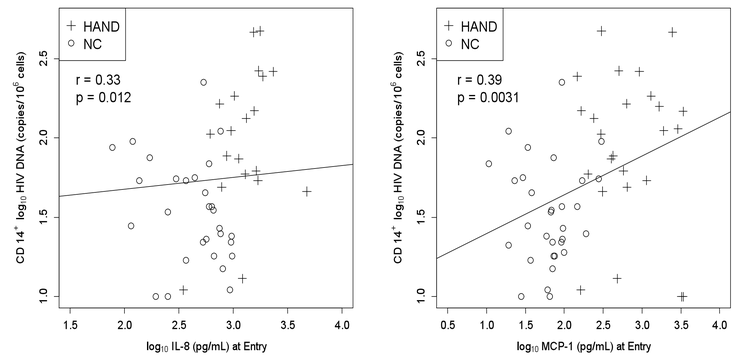
- IL-8 secretions were significantly increased in HAND individuals after one year on cART and although not significant, MCP-1 secretions were higher in HAND individuals after one year on cART



- However, significance is reached if the HAND individuals that become NC are separated out of the NC group after one year on cART



- CD14+ PBMC HIV DNA levels correlated to IL-8 and MCP-1 secretions at Entry



Discussion

- cART effectiveness is demonstrated by the improvement in cognition of 18 individuals from HAND to NC after one year on cART
- Despite improvements in cognition after being on cART for one year, the NC individuals that were originally diagnosed with HAND at entry still had higher levels of IL-8 and MCP-1 secretions when compared to the NC group
- The higher levels of cytokine production suggests that cART does not completely impede inflammation and the presence of viral reservoirs, such as monocytes, contribute to the persistence of inflammation

Conclusion

- Of the 10 cytokines measured in CD14+ PBMC tissue culture supernatants, IL-8 and MCP-1 were found to be higher in HAND individuals at entry and after one year on cART
- The difference in MCP-1 secretions after one year on cART between NC and HAND becomes significant when the NC individuals that were originally HAND are separated out into their own group
- CD14+ PBMC HIV DNA levels correlated to both IL-8 and MCP-1 secretions at entry, which indicate viral reservoirs contribute to inflammation that can result in HAND
- The secretion of these cytokines from monocyte reservoirs may play an important role in chronic inflammation and the continued presence of HAND regardless of cART

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