HIV-associated cognitive disorders: Scientific discoveries through international collaborations in Thailand
Valcour, Victor

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CHAPTER 5
HIV DNA IN CIRCULATING MONOCYTES AS A MECHANISM TO DEMENTIA AND OTHER HIV COMPLICATIONS

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HIV DNA IN CIRCULATING MONOCYTES AS A MECHANISM TO DEMENTIA AND OTHER HIV COMPLICATIONS

Victor Valcour\textsuperscript{1,2}, Bruce Shiramizu\textsuperscript{1}, and Cecilia M. Shikuma\textsuperscript{1}

\textbf{AFFILIATIONS}
\textsuperscript{1} Hawaii Center for AIDS, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA
\textsuperscript{2} Memory and Aging Center, Department of Neurology and the Division of Geriatric Medicine, Department of Medicine, University of California, San Francisco, San Francisco, California, USA

\textbf{ABSTRACT}

It is broadly accepted that HIV DNA in lymphoid and myeloid cells persists despite combination antiretroviral therapy. Recognized as the Achilles heel to HIV eradication, the role of these peripheral reservoirs in HIV morbidity is less well developed. The burden of HIV DNA in peripheral mononuclear cells is linked to HIV disease outcomes such as time to AIDS diagnosis, survival, and CD4 T-lymphocyte counts. Monocytes are a minor HIV DNA reservoir, and the burden of HIV DNA in these cells appears to be linked to dementia, suggesting that residual infection in this subset is linked to tissue-related HIV complications. Since monocytes are likely involved in trafficking virus to the brain, there is a strong mechanistic link underlying this discovery. Herein, we summarize our current understanding of monocyte HIV DNA and central nervous system dysfunction in humans. We present a model to understand these relationships and suggest possible treatment approaches to be tested.

\textbf{INTRODUCTION}

HIV-associated dementia (HAD), the most severe form of HIV-related cognitive dysfunction, is characterized by abnormalities in motor skills (slowed movements, abnormal gait, hypertonia), behavior (apathy, irritability, emotional lability), and cognitive function (attention, concentration, memory, information processing, language).\textsuperscript{1} Prior to widespread use of highly active antiretroviral therapy (HAART), the prevalence of HAD was 20–30% among patients with advanced HIV and low CD4 lymphocyte counts. The widespread availability of HAART led to a marked decline in the reported incidence of HAD to 10/1000 person-years in 1996–1998.\textsuperscript{2}

The characteristics of HIV-related cognitive dysfunction have also changed. Cognitive complications are noted with higher CD4 lymphocyte counts and milder degrees of cognitive impairment are more typical.\textsuperscript{3} Paradoxically, while HAD incidence has decreased, prevalence may be rising as individuals on HAART live longer. The HAART-era prevalence of HAD or a milder variant referred to as mild neurocognitive disorder (MND) may be as high as 37%.\textsuperscript{2,4,5} Fluctuation in disease course with a waxing and waning pattern has now been described.\textsuperscript{3,6} Taken together, these findings highlight the failure of HAART to universally eradicate cognitive impairment. The identification of mechanistic underpinnings has been disappointing, leading some to believe that much of the impairment is due to comorbid illnesses or pre-HAART (permanent) brain injury.

\textbf{THE NEUROIMMUNOLOGY OF HIV}

Human immunodeficiency virus (HIV) encephalitis is the classically described substrate of HIV brain injury, characterized by gliosis, microglial nodules, perivascular macrophage accumulation, and the presence of multinucleated giant cells.\textsuperscript{7} These findings are associated with immune activation and inflammation seemingly out of proportion to the amount of HIV virus present in the brain.\textsuperscript{8-10} Although viral particles, such as nef, gp120, and tat, are neurotoxic in vitro, the mechanisms of brain injury likely involve a significant contribution from indirect immunological processes, including a
prominent role for inflammatory pathways mediated by cells of the monocyte/macrophage lineage.\textsuperscript{10-14}

On the basis of evidence from animal models and autopsy data, the clinical onset of neurological disease and its acceleration as immune function fails, directly relate to the dysregulation and accumulation in the central nervous system (CNS) of activated perivascular macrophages, some of which are infected.\textsuperscript{8} The number of inflammatory macrophages rather than the concentration of virus is the best indicator of neural damage and cognitive deterioration in simian immunodeficiency virus (SIV)-encephalitis, and the majority of the SIV in the CNS is within these perivascular macrophages and not in parenchymal microglia.\textsuperscript{8,9,15,16} Recent studies suggest that the accumulation of perivascular macrophages in late-stage disease is due to increased trafficking of peripheral monocytes into the CNS.\textsuperscript{8,16-18} A unifying hypothesis is proposed that HIV encephalitis following virally induced failure of the immune system is primarily a disease resulting from blood-borne activated macrophages capable of stimulating inflammatory responses in the CNS.

The phenotype of the perivascular macrophage is similar to that of a minor monocyte population found in the peripheral blood defined by the co-expression of CD14 and CD16 and/or CD69.\textsuperscript{19} Populations of CD14+/CD16+ and CD14+/CD69+ monocytes are expanded in HIV-infected patients and correlate to HAD.\textsuperscript{20-22} On the basis of these and other such data, it is hypothesized that critical events initiating the development of dementia occur outside of the brain.\textsuperscript{15} This concept is further supported by the finding that sequences of the HIV viral gp160 gene, which encode the highly variable HIV envelope protein, taken from the deep white matter of the brain in an individual with HAD were more closely related to sequences from the bone marrow and to sequences from blood monocytes taken 5 months earlier than to those from other tissues.\textsuperscript{15}

These findings are consistent with leading theories of encephalitis development in HIV.\textsuperscript{10,15} Here, peripherally infected monocytes, upon meeting the brain microvascular endothelium, are able to self-initiate transmigration to the brain because of their activated state. These cells then become perivascular macrophages and may also transfec other resident cells (astrocytes, microglia). The result is a proinflammatory environment characterized by cellular activation, heightened secretion of cytokines and chemokines, and increased oxidative stress ultimately contributing to neuronal dysfunction and HAD (Figure. 1).

**PERIPHERAL RESERVOIRS OF HIV DNA BEFORE AND AFTER HAART**

The retrovirus, HIV, enters human immune cells through CD4 and the co-receptors CCR5 or CXCR4, and through reverse transcription, incorporates into host DNA.\textsuperscript{23} Subsequent transcription results in rapid and extensive HIV RNA production, cell lysis, and the prototypical exponential rise of HIV RNA in the bloodstream. Plasma viremia becomes life-long in the absence of treatment and immunodeficiency ensues in the vast majority of individuals. Because of the archival nature of the immune system, a minor subset of activated and infected CD4+ T-lymphocytes may become memory T-cells that are quiescent with little to no production of virus unless stimulated.\textsuperscript{24,25} It is postulated that monocytes are also a source of residual HIV DNA, likely arising from quiescent and infected cells of the myeloid precursor lineage in bone marrow;26–31 HIV DNA within monocytes may exist integrated into the host genomic
DNA or as nonintegrated circular forms and both resistant and wild-type viruses have been demonstrated.32,33 Among individuals on HAART, the CD16+ subset of monocytes appears more susceptible than other monocytes to HIV infection and preferentially harbor HIV DNA.34,35 CD16+ monocytes produce high levels of chemokines, increasing the susceptibility of resting T cells to HIV infection.36 Macrophages are generally resistant to the cytopathic effects of the virus and may persist in the tissues for a long period of time despite suppression of plasma HIV RNA.37 In one study, levels of HIV DNA in purified monocyte-depleted peripheral mononuclear cells were identified in all cases both prior to and 24 months following effective HAART.38 In contrast, HIV DNA was detected in monocytes from all naive cases and 12/34 (35%) of treated cases.

**HIV DNA AND DISEASE**

The level of HIV DNA in PBMCs predicts HIV events, including rate of decline in CD4 lymphocyte count, time to AIDS diagnosis, and dementia. Early reports identified a relationship to poor antiretroviral response and, more recently, to virological failure.39,40 HIV DNA predicts progression to AIDS in the SEROCO cohort, independently of plasma HIV RNA and CD4 counts and disease progression in the PRIMO Cohort.41,42 In one study, among HAART-naive patients, PBMC HIV DNA but not monocyte HIV DNA inversely correlated to CD4 count and PBMC HIV DNA correlated to high (>30,000 copies) compared with low HIV RNA.38 This finding suggests that HIV DNA within lymphocytes, rather than within monocytes, may have more relevance to primary non-tissue related HIV disease variables such as CD4 lymphocyte reduction.

In the setting of chronic HIV infection, PBMC HIV DNA has been identified as a correlate to HAD. In a cross-sectional study done in our laboratory, the median HIV DNA level among individuals with HAD were roughly 20 times higher than the median level among individuals with normal cognition.43 This association remained significant among those individuals with undetectable plasma HIV RNA. PBMC HIV DNA is also higher in individuals with milder cognitive impairment although not to the extent seen in individuals with HAD.44 Further analyses by cellular subsets revealed that the difference in HIV DNA by cognitive category is related specifically to the amount of HIV DNA within the activated mono-

![Figure 2. HIV DNA content in various monocyte blood cell subsets in individuals with HAD vs. normal cognition (NC). (A) HIV DNA is higher in individuals with HIV-associated dementia (HAD) (left) compared with individuals with NC in the CD14+/CD16+ subset. (B) and (C) In CD14+/CD16- (B) and CD14+ (C) subsets, the amount of HIV DNA does not differ between individuals with HAD (left) and individuals with NC.](image-url)
cytes (CD14+/CD16+) subset rather than in non-activated monocytes (CD14+/CD16neg) or in CD14neg cells, which includes the lymphocyte pool (Figure 2).  

Levels of monocyte HIV DNA in individuals with HAD remained persistently high over time when compared with individuals with normal cognition. In a cohort of HIV-infected individuals in Thailand naive to HAART, baseline and 48-wk after HAART treatment, monocyte HIV DNA levels strongly correlated to concurrent cognitive performance irrespective of plasma HIV RNA and CD4 lymphocyte counts. At 48 week, monocyte HIV DNA was below the level of detection of our assay (10 copies/106 cells) in 15/15 non-HAD compared with only 4/12 HAD cases, despite undetectable plasma HIV RNA in 26/27 cases (Figure 3). These findings have important implications for HAART-era cognitive impairment. Although published data indicate that mild cognitive impairment remains prevalent in patients successfully treated with HAART, few investigators have identified HIV-specific mechanistic links or markers, leading some to hypothesize that the cognitive findings represent inactive disease. However, HIV DNA in circulating monocytes remains elevated in some individuals despite HAART, and the inability to clear this peripheral reservoir is more frequent among individuals with dementia, suggesting that the HAART-era cognitive impairment may, at least in part, be due to ongoing injury. Given that monocytes likely traffic to the CNS resulting in inflammation and viral seeding, it is plausible that HIV infection of these monocytes may contribute to CNS injury, even in the era of HAART.

MONOCYTE HIV DNA AND OTHER CHRONIC COMPLICATIONS OF HIV

The role of monocyte HIV DNA in other HIV-associated complications is less clear. Given the highly pro-inflammatory nature of HIV-infected activated monocytes and the presence of macrophages in various tissues, it is reasonable to postulate a role of HIV DNA in association with other chronic complications of HIV. High levels of HIV DNA have been hypothesized to play a potential role in unintentional weight loss, as well as in lipoatrophy. High levels of macrophages are found within subcutaneous fat tissue of HIV-infected patients with HIV-associated lipoatrophy. Moreover, high levels in fat tissue of pro-inflammatory cytokines (TNF, IL-6, IL-8, IL-12, IL-18) are found in adipose tissue that correlate significantly with adipose tissue macrophage content, suggesting that the inflammatory cytokines originated from these macrophages. High rates of metabolic dysregulation, including insulin resistance, characterize HIV-infected individuals on HAART. In the general population, monocyte/macrophage and their proinflammatory cytokines have been demonstrated to play significant pathogenic roles in obesity and in insulin resistance-related disease processes, as well as in vascular activation and inflammation. Thus, it is likely that monocyte/macrophage-mediated immune activation and inflammation increase risk for such complications in the HIV-infected population.

The transmigration of monocytes into the arterial vessel wall initiates the development of atherosclerosis. Such transmigration is enhanced by the inflammation associated with HIV infection, much of which may originate from monocyte/macrophages. Once in the arterial vessel wall, monocytes...
are transformed into lipid-laden macrophages or “foam cells,” which form the lipid-rich core of atheromatous plaques. HIV-infected macrophages may have a role in the development of foam cells since HIV impairs ATP binding cassette transporter A1 (ABCA1)-dependent cholesterol efflux from macrophages. This condition is likely to be highly atherogenic as the resulting deficit in reverse cholesterol transport can be expected to greatly enhance the accumulation of cholesterol within these cells and increase the development of foam cells. The cholesterol efflux impairment in HIV-infected macrophages and the known persistence of these long-lived HIV-infected pools despite HAART could partially explain the increase in cardiovascular disease seen in the era of HAART.

**POTENTIAL APPROACHES TO MODULATING THE MAGNITUDE OF HIV DNA IN PERIPHERAL RESERVOIRS**

No validated therapeutic approaches to eradication of the peripheral HIV DNA reservoir exist. Control of HIV viremia may diminish this peripheral intracellular reservoir over time and eradication may be potentially modifiable with early and aggressive treatment with HAART. Recently, a group from France noted that the mean HIV DNA burden is substantially lower in individuals treated during primary infection compared with those initiating treatment during the chronic phase of disease. It is not known, however, if both lymphocyte and monocyte HIV reservoirs are equally affected. Since it is increasingly recognized that the earliest events in HIV infection may set the stage for disease course, it is intriguing to hypothesize that early and intensive treatment may limit the magnitude of the monocyte reservoir.

Although necessarily speculative, other treatment approaches may exist within the confines of currently available therapies. Antiretroviral choice that may be considered “monocyte-directed” deserve evaluation. CCR5 antagonists may have some enhanced efficacy as part of ‘monocyte-directed therapy.’ Both CCR5 and CXCR4 may serve as chemokine receptors for entry of HIV into lymphocytes while CCR5 is the major co-receptor used for HIV entry into macrophages and into brain microglia cells. To date, clinical studies have not demonstrated the efficacy of this “monocyte directed” approach.

Some have noted that NRTIs are more effective in eradicating virus in monocytes/macrophages than in CD4+ cells, owing to the quiescent nature of monocytes/macrophages and a low endogenous nucleotide pool, enabling the triphosphate forms of the drugs to more successfully compete against the endogenous pool for binding to the HIV reverse transcriptase. Of the newer nucleoside/ nucleotide reverse transcriptase inhibitors, tenofovir has potent anti-HIV activity in monocytes, greater than that observed in CD4 lymphocytes. This has been attributed to its mono-phosphorylated structure, reducing the requirement for intracellular phosphorylation to its active triphosphate form. Consequently, tenofovir has been proposed for post-exposure prophylaxis, due to its enhanced anti-HIV effects in tissue-laden monocyte-derived cells (Langerhan cells). It should be noted that tenofovir does not effectively cross the blood-brain barrier, a factor that may raise some concern with the use of tenofovir in individuals with HIV-associated cognitive dysfunction. However, it has also been postulated that the efficacy of NRTIs in the CNS may be greater than suggested by BBB dynamics and CSF concentrations because of the previously mentioned enhanced intracellular activity in monocytes/macrophages and the critical role of these cells in the CNS. In vitro studies suggest that protease inhibitors (PI), in general, have decreased potency against monocytes. PI concentrations needed to inhibit monocyte HIV RNA production exceed that commonly seen in HIV treatment, although ritonavir boosting appears to overcome much of this issue.

Other “monocyte directed” therapeutic approaches are under investigation, including polyamine biosynthesis inhibitors, a class of drugs shown in vitro to selectively kill activated monocytes from patients with HIV dementia and advanced HIV disease. Facilitation of antiretroviral medication carriage across the blood-brain barrier by use of medication containing nanoparticles loaded into bone marrow macrophages has successfully been explored in an HIV encephalitis rodent model.
Unlike T-lymphocytes, HIV infected macrophages do not undergo cell death, even when exposed to toxic conditions, thus serving as long-living viral reservoirs in various tissues. Studies of molecular and cellular mechanisms involved in this cytoprotective effect in primary human macrophages indicate that the P13K/Akt pathway is a key contributor to this effect. P13K/Akt inhibitors were recently shown in a cell model to reverse key cellular events typically observed in cell survival activation and result in reduced HIV production. Finally, murine studies have been performed using erythrocytes loaded with a new heterodinucleotide (3TCpPMPA) comprising the drugs lamivudine and tenofovir and modified to increase their phagocytosis by macrophages in an effort to more effectively deliver these antiretroviral medications into macrophages.

**SUMMARY**

Detectable levels of HIV DNA in circulating monocytes are seen in a subset of individuals on HAART. The inability of potent antiretroviral therapy to successfully eradicate HIV from this peripheral reservoir is associated with dementia and may, at least in part, account for the continued prevalence of neurocognitive impairment in HIV-infected individuals in the HAART era. Recent studies have led to a better understanding of the unique characteristics and importance of this cellular reservoir in the pathogenesis of HIV dementia. Because effective eradication of this reservoir is unlikely to occur with use of antiretroviral therapy as currently practiced, continued research into novel therapeutic approaches to eradication of this cellular reservoir is desperately needed.

**REFERENCES**


KEY WORDS
AIDS dementia complex, antiretroviral therapy, lipodystrophy

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Dr. Valcour is a consultant for GlaxoSmithKline, Abbott, and Merck; Dr. Shikuma receives HIV research and training funding support from Merck, Gilead, and Pfizer, and is a consultant and on the speakers bureau for GlaxoSmithKline.

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