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Monocytes Mediate HIV Neuropathogenesis: Mechanisms that Contribute to HIV Associated Neurocognitive Disorders

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Abstract

HIV infected people are living longer due to the success of combined antiretroviral therapy (cART). However, greater than 40-70% of HIV infected individuals develop HIV associated neurocognitive disorders (HAND) that continues to be a major public health issue. While cART reduces peripheral virus, it does not limit the low level, chronic neuroinflammation that is ongoing during the neuropathogenesis of HIV. Monocyte transmigration across the blood brain barrier (BBB), specifically that of the mature $CD14^+CD16^+$ population that is highly susceptible to HIV infection, is critical to the establishment of HAND as these cells bring virus into the brain and mediate the neuroinflammation that persists, even if at low levels, despite antiretroviral therapy. $CD14^+CD16^+$ monocytes preferentially migrate into the CNS early during peripheral HIV infection in response to chemotactic signals, including those from CCL2 and CXCL12. Once within the brain, monocytes differentiate into macrophages and elaborate inflammatory mediators. Monocytes/macrophages constitute a viral reservoir within the CNS and these latently infected cells may perpetuate the neuropathogenesis of HIV. This review will discuss mechanisms that mediate transmigration of $CD14^+CD16^+$ monocytes across the BBB in the context of HIV infection, the contribution of these cells to the neuropathogenesis of HIV, and potential monocyte/macrophage biomarkers to identify HAND and monitor its progression.

Keywords

HIV; $CD14^+CD16^+$ Monocyte; Chemokine; BBB; Neuroinflammation

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Introduction

HIV has become a chronic infection with the advent of combined antiretroviral therapy (cART). Despite the success of this therapy, the brain remains a viral reservoir [1]. HIV enters the CNS within 14 days after primary infection [2] and initiates events that result in cognitive, motor, and behavioral deficits, collectively known as HIV associated neurocognitive disorders (HAND) or neuroAIDS [3-5], in approximately 40-70% of HIV infected individuals. NeuroAIDS encompasses a spectrum of dynamic disorders that greatly impact the quality of life of HIV infected people, and thus is a significant public health concern.

One major mechanism that contributes to HAND is the transmigration of monocytes, specifically the $CD14^{+}CD16^{+}$ subpopulation, across the blood brain barrier (BBB) into the CNS. This process initiates and propagates viral infection of the brain and is mediated by interactions between monocytes, cells of the BBB, and chemotactic gradients that bring these leukocytes into the CNS. Despite effective antiretroviral therapy, monocytes are believed to continue to enter into the CNS of HIV infected individuals resulting in low level, ongoing, chronic neuroinflammation. This review will focus on monocytes as a central mediator of HAND and the mechanisms that contribute to the transmigration of both infected and uninfected cells into the brain of HIV infected individuals.

Monocyte Heterogeneity

NeuroAIDS occurs and persists due, in part, to the entry of a mature monocyte population into the brain parenchyma. These cells become infected in the peripheral blood and bring the virus into the CNS [6]. Monocytes are heterogeneous and populations exist with distinct surface markers, extent of maturation, granularity, and functions [7-10]. CD14, the LPS receptor, is the most widely used surface marker to identify human peripheral blood monocytes. CD16, the FcγIII receptor, is also expressed on some monocytes. The majority of monocytes from healthy individuals express CD14 with no CD16. These $CD14^{++}CD16^{-}$ cells comprise 90-95% of monocytes [9-11]. Monocytes that express both CD14 and CD16 are believed to be a more mature subset [12, 13]. There is additional heterogeneity within the $CD14^{+}CD16^{+}$ monocyte population determined by the extent to which surface CD14 and CD16 are expressed [9]. Some $CD14^{+}CD16^{+}$ monocytes have high expression of CD14 ($CD14^{++}CD16^{+}$), while others express less surface CD14 and more CD16 ($CD14^{+}CD16^{++}$), as determined by flow cytometry. We and others characterized phenotypic and functional differences among these monocyte subsets [11, 14-17].

In 2010, nomenclature was developed by the Nomenclature Committee of the International Union of Immunological Societies (NC-IUIS) to distinguish among the monocyte populations [18]. In this nomenclature, the predominant $CD14^{++}CD16^{-}$ population was termed “classical monocytes”. The terminology used to denote the remaining $CD14^{+}CD16^{+}$ subsets included “intermediate monocytes” to describe the $CD14^{++}CD16^{+}$ population and “nonclassical monocytes” for the $CD14^{+}CD16^{++}$ cells. The NC-IUIS defined parameters to distinguish the relative amounts of CD14 and CD16 expressed among these three monocyte subsets by flow cytometry based upon isotype matched negative control antibodies [18]. Despite this proposed protocol, there remains no standard gating strategy for flow

cytometric analysis of monocyte subsets [19]. The variability inherent in primary human cells contributes to the difficulty in delineating these populations. The percentage of the nonclassical and intermediate monocyte subsets that constitute $CD14^{+}CD16^{+}$ cells is variable, and was shown to fluctuate even in the same individual from day to day [19].

When monocytes are analyzed by flow cytometry for surface CD14 and CD16, the two markers demonstrate a continuum of expression levels rather than distinct, isolated populations [16]. The intermediate $CD14^{++}CD16^{+}$ monocytes have been described to be in transition from classical to nonclassical monocytes and may therefore not constitute a unique population [19]. In support of this, it has been suggested that there is a developmental relationship among the monocyte subsets. As monocytes circulate in the peripheral blood, they mature from the $CD14^{++}CD16^{-}$ population and acquire CD16 to become $CD14^{++}CD16^{+}$ and ultimately mature further into $CD14^{+}CD16^{++}$ cells [14, 20, 21]. For this review, we term all monocytes that express surface CD14 and CD16 as $CD14^{+}CD16^{+}$, regardless of the amount of each antigen present.

As the $CD14^{+}CD16^{+}$ monocyte population is present in such few numbers in the blood of healthy individuals, analyses of the functions and cell surface markers specific to this population are difficult. Our laboratory therefore developed a culture system that enables the enrichment of this $CD14^{+}CD16^{+}$ monocyte subset and characterized markers associated with monocyte maturation, susceptibility to HIV infection, and transmigration across the BBB [11]. This culture method consists of isolating peripheral blood mononuclear cells (PBMC) by Ficoll-Pacque density gradient, followed by obtaining monocytes by CD14 positive selection. The isolated monocytes are then cultured nonadherently in Teflon flasks to prevent adherence-mediated differentiation into macrophages [22, 23]. The cells are cultured in the presence of the monocyte/macrophage growth factor M-CSF for three days, as this is the period of time during which monocytes circulate in the blood prior to entering tissues to become macrophages [24]. While there are few $CD14^{+}CD16^{+}$ monocytes after initial isolation from PBMC, the numbers of these cells are significantly increased using this culture method [10, 11, 25].

HIV Infection of $CD14^{+}CD16^{+}$ Monocytes

The $CD14^{+}CD16^{+}$ monocyte population is critical to the neuropathogenesis of HIV infection. This subset constitutes only 5-10% of peripheral blood monocytes in healthy individuals, but their percentage can increase up to ∼40% in HIV infected people [26-28]. The mature monocyte population is more permissive to viral entry and replication than $CD14^{+}CD16^{-}$ cells and continues to harbor HIV, even with successful cART [29-35]. $CD14^{+}CD16^{+}$ monocytes express higher amounts of the HIV coreceptor CCR5 as compared to $CD14^{+}CD16^{-}$ cells [29] which facilitates their productive infection both *in vitro* and *in vivo*[11, 29, 30, 36-38].

It was previously believed that monocytes were refractory to HIV infection. This reported lack of susceptibility to HIV might have been because most *in vitro* infection studies were performed with monocytes isolated from healthy individuals that have few $CD14^{+}CD16^{+}$cells. Monocyte susceptibility to infection by many viruses, including HIV, is maturation dependent and increases as these cells mature [11, 39-43].

Using our *in vitro* monocyte maturation culture system to obtain large numbers of $CD14^+CD16^+$ cells, we demonstrated that $CD14^+CD16^+$ monocytes are highly permissive to HIV infection and express more surface CCR5 than their $CD14^+CD16^-$ counterparts [11] (unpublished data), mirroring what is seen *in vivo*. In these studies freshly isolated monocytes, comprised of only 5-10% $CD14^+CD16^+$cells, had minimal virus production, as measured by p24 ELISA. In contrast, mature $CD14^+CD16^+$monocytes obtained after our culture method were highly infected by HIV [11]. To determine novel host proteins that may facilitate the preferential infection of $CD14^+CD16^+$ monocytes, we performed microarray analyses using RNA from cells upon initial isolation and after 3 days of nonadherent culture. There were several genes identified by the microarray whose protein products have been shown to interact with viral proteins, including fibronectin and syndecan-2 [11]. Fibronectin and syndecan-2 are believed to be important for viral entry as they act as attachment receptors that enable the virus to interact with the cell. These proteins have been implicated in facilitating entry of HIV as well as of other viruses, including herpes simplex, into many cell types[44-49]. We are currently determining the role of these proteins in HIV infection of $CD14^+CD16^+$ monocytes.

Monocytes, HIV Neuropathogenesis, and Neuroinflammation in the Era of cART

Once $CD14^+CD16^+$ monocytes become infected with HIV, they contribute significantly to the establishment of a viral reservoir within the CNS. Unlike CD4 T lymphocytes, cells of the monocyte lineage are not susceptible to the cytopathic effects of HIV [50-54]. Monocytes that were infected in the periphery can produce infectious virus as they enter into the CNS and when they differentiate into macrophages. These differentiated macrophages remain as a source of virus because they are very long-lived cells [55, 56]. In support of this, $CD14^+CD16^+$ cells positive for HIV antigens have been identified in the perivascular space and within the brain parenchyma of HIV infected individuals, indicating that these cells contribute to the productive infection of the CNS [57-59].

Transmigration of HIV infected monocytes into the brain contributes to chronic inflammation and immune activation within the CNS (Figure 1). The virus released by monocytes promotes infection of resident CNS cells including microglia, macrophages, and a small percentage of astrocytes [60, 61]. Neurons are not productively infected by HIV, but are impacted by the consequences of CNS infection. Infected cells within the brain release cytokines, chemokines, and neurotoxic viral proteins, including tat and gp120, which activate surrounding cells [62]. Activated microglia, macrophages, and astrocytes also produce inflammatory mediators including TNF-α, IL-1β, and IL-6 and the chemokines CCL2 and CXCL12, that recruit additional $CD14^+CD16^+$ monocytes into the CNS contributing to neuroinflammation [63]. The infected and activated CNS cells elaborate neurotoxic host factors including arachidonic acid, quinolinic acid, and nitric oxide[62]. These mediators can dysregulate BBB permeability and astrocytic glutamate uptake. All of these processes that occur during CNS infection can ultimately result in the neuronal damage and loss associated with HAND [64] (Figure 1).

Prior to antiretroviral therapy the presence of monocytes and macrophages in the CNS was strongly associated with HAND. In the antiretroviral era, the question now arises whether

monocytes remain relevant to HIV CNS disease. Some studies found that inflammation and monocyte/macrophage activation persist in the CNS despite cART [65-72], while others have not [73]. When detected, neuroinflammation is prevalent even with the milder forms of cognitive impairment [74, 75]. The neuroinflammation that occurs may be present at very low levels, less than those associated with full blown encephalitis [71]. The fact that inflammation and monocyte/macrophage activation often occur despite antiretroviral therapy, regardless of their extent, increases the risk for the development of cognitive deficits.

A gene expression array demonstrated that there may be two forms of cognitive impairment that occur in the present era of antiretroviral therapy driven by differing pathophysiologies [76]. “Type I” impairment is characterized by HIV encephalitis and high CNS viral loads which persist in some individuals despite cART. The cognitive deficits associated with this form of impairment may benefit from decreasing replication in the brain. “Type II” impairment has low viral loads in the brain and no encephalitis, but is instead characterized by dysregulated endothelial signaling, suggesting that vascular stress may be contributing to these cognitive deficits.

Antiretroviral treatment itself may also contribute to differing forms of HAND in HIV infected individuals. Genome-wide microarray analysis was performed with RNA isolated from brain tissue of people with HAND who were either treated with cART or were naïve to therapy [77]. In this study, monocyte/macrophage markers CD14 and CD68 were decreased with cART treatment, but remained present. This may be indicative of what occurs during “Type II” impairment. There were 100 genes involved in adaptive immunity and interferon related pathways that remained dysregulated in both the treated and untreated groups [77]. A better understanding of these common pathways as well as the mechanisms that contribute to neuroinflammation and monocyte/macrophage activation are necessary to address further the HIV associated cognitive deficits that occur in the antiretroviral era.

Chemokines in the Context of HAND: CCL2 and CXCL12

The chemotactic signals that facilitate transmigration of $CD14^{+}CD16^{+}$ monocytes across the BBB contribute to the initial entry of HIV infected monocytes into the CNS and the subsequent low level neuroinflammation that persists despite cART. Chemokineelaboration by resident CNS cells is dysregulated during HIV infection[78]. Two chemokines implicated in the neuropathogenesis of HIV are monocyte chemoattractant protein-1, CCL2, and stromal cell-derived factor-1, CXCL12. Elevated levels of these chemokines in the CNS of HIV infected people suggest their importance in the recruitment of cells from the periphery across the BBB and may promote the initiation and/or the establishment of HAND.

In the pre-cART era, elevated CNS CCL2 was specifically associated with HIV associated dementia (HAD), the only characterized cognitive deficit at that time. This chemokine was upregulated in the brain tissue of HIV infected individuals with dementia, but not in those without cognitive deficits [79]. In the current therapeutic era, CCL2 remains increased in the CSF of HIV infected, cART treated people with HAND irrespective of the severity of cognitive impairment[80]. More strikingly, even aviremic HIV seropositive individuals on suppressive cART show a significant increase in CSF CCL2 levels in comparison to healthy,

seronegative counterparts [80-82]. This suggests that many cART-treated, HIV infected individuals have CCL2-mediated monocyte influx into the CNS, and that although there may not yet be any manifestations of cognitive deficits, these individuals are at risk for HAND.

Many cell types in the CNS produce CCL2, including astrocytes and macrophages, as part of normal immune trafficking. Upon infection with HIV or simian immunodeficiency virus (SIV), these cell types secrete even higher amounts of CCL2[83]. The chemokine can be elaborated not just in response to viral infection but also by exposure to viral proteins, such as tat, which remains present in the CNS despite successful cART[84-86]. HIV tat induces astrocytic CCL2 production [87, 88], as does tat treatment of human microglia [89]. However, the effects of CCL2 may also be protective depending on the kinetics and localization of its expression within the CNS[90].

CXCL12 is also implicated in mediating the initiation and progression of HAND. CXCL12, like CCL2, is increased in the CSF during HIV infection and during other CNS inflammatory disorders, including multiple sclerosis [91-97]. CXCL12 mediates its effects by signaling through its receptor CXCR4, although CXCR7 has been identified as an additional receptor for this chemokine [98]. CXCR7 and CXCR4 can heterodimerize and facilitate CXCL12-mediated chemotaxis [99]. It remains unclear whether CXCR7 contributes to the establishment or progression of neuroAIDS.

While much focus has been on this chemokine as a T cell chemoattractant, our laboratory previously showed that CXCL12 is also chemotactic for monocytes [100]. We now demonstrate that the specific $CD14^+CD16^+$ monocyte population transmigrates across our *in vitro* model of the BBB[101, 102] in response to CXCL12 (Figure 2). Interestingly, the number of $CD14^+CD16^+$ cells that cross our barrier model in response to CXCL12 is similar to that to CCL2. As CXCL12 is increased in the brain during HIV infection, it, too, may mediate the migration of mature monocytes that are highly susceptible to HIV infection into the CNS of seropositive individuals, contributing to the seeding of the CNS with virus and to neuroinflammation. Prior to antiretroviral therapy, CXCL12 expression in neurons of the basal ganglia was associated with HIV encephalitis [103, 104], but this has not been examined in the post-cART era.

CXCL12 is produced by astrocytes, macrophages, and neurons. Astrocytes treated with conditioned media from HIV infected monocyte derived macrophages (MDM) significantly increased their production of CXCL12[105]. When MDM were treated with IL-1β siRNA, the astrocytic production of CXCL12 caused by MDM conditioned media was inhibited, suggesting that the release of IL-1β by HIV-1 infected macrophages contributed to the increased production of CXCL12 by astrocytes [105]. Additionally, *in vitro* treatment with tat induced neuronal expression of CXCL12, further implicating the importance of this viral protein in promoting the production of chemokines that recruit monocytes into the CNS [103].

Mechanisms of Monocyte Transmigration Across BBB

The events that lead to the establishment of neuroAIDS occur soon after peripheral HIV infection. HIV enters the CNS as early as 8-14 days post-infection, before most are even aware of their HIV status [106, 107]. This suggests that many HIV infected individuals have an established viral reservoir in the brain prior to the initiation of cART. Additionally neuroinflammation and neuronal injury, which ultimately lead to the deficits associated with HAND, can occur during the first weeks of HIV infection [2, 106, 108].

During the acute phase of HIV, when viral load is high, HIV infected monocytes may transmigrate across the BBB in large numbers in response to chemokines constitutively present in the brain. We propose that high numbers of infected cells transmigrating across the BBB, and their subsequent entry into the CNS parenchyma, initiates and precedes the establishment of HAND. These highly infected cells, along with CNS viral infection, may cause transient breaching of the barrier [102]. Upon the initiation of antiretroviral therapy, as virus production is reduced, these alterations to the barrier will likely resolve and BBB integrity will be restored. Monocyte influx into the brain that occur as a result of CNS infection and activation, in response to elevated levels of chemokines, will continue even when viral loads are low to undetectable. In this chronic phase of HIV infection, we hypothesize that both uninfected and low level or latently infected monocytes will transmigrate into the CNS and promote chronic neuroinflammation that participates in HAND in the post-cART era[25].

The BBB consists primarily of brain microvascular endothelial cells (BMVEC) and astrocytes and is a selectively permeable barrier that separates the periphery from the CNS [109-111]. Tight junctions between the BMVEC act as a dynamic barrier that inhibits paracellular transit into the brain [112-114]. Immune cell entry into the CNS is highly regulated. Baseline immune surveillance by leukocytes, including monocytes, across the BBB occurs at a much lower rate than that in peripheral organs [115-117].

Transmigration is a well-coordinated, multistep process that involves the entry of cells from the lumen of blood vessels into tissues [118]. Much of the understanding of transmigration across the BBB came from studies of monocyte entry into the peripheral vasculature. There is less information regarding the steps that promote and facilitate monocyte entry across the specialized cells of the BBB. Many of the mechanisms that mediate transmigration across the peripheral vasculature and the BBB are conserved[10], but there may also be distinct components specific to the CNS. The steps in transmigration include monocyte capture from the circulation onto the endothelium, rolling, firm arrest, intravascular crawling, and lastly diapedesis through the BMVEC and basement membrane into the CNS [10, 119].

Diapedesis is the final step of transmigration and involves adhesion molecules and tight junction proteins, which we designate collectively as junctional proteins. Some of these include JAM-A, ALCAM, CD99, and PECAM-1 [10, 120]. These proteins are present on both the BMVEC and the transmigrating monocyte and interact to shepherd the monocyte across the BBB. The junctions of the endothelium open and interact hetero- and homotypically with the junctional proteins on monocytes in a zipper-like manner. The endothelial cell junctions then quickly close as the monocyte passes through. This entire

process is complete within 90 seconds [121]. Monocyte diapedesis can occur between the BMVEC (paracellular transmigration) or through the endothelium (transcellular transmigration) [122]. Transcellular transmigration across the peripheral vasculature occurs at a frequency of 9-30% [123]. Although the physical route across the endothelium differs between these two mechanisms, many of the same junctional proteins are conserved between paracellular and transcellular passage [124]. Therapeutically targeting the steps involved in monocyte transmigration may help limit viral entry into the CNS and the neuroinflammation that occur during neuroAIDS.

Using our *in vitro* model of the human BBB, we demonstrated that $CD14^+CD16^+$ monocytes preferentially transmigrated across the BBB in response to CCL2[11] as well as CXCL12. This model consists of culturing human BMVEC and human astrocytes on opposite sides of a tissue culture insert with 3 μm pores [101, 102]. The cocultures grow to confluence over 3 days. During this time the astrocytic end feet processes penetrate the insert to contact the BMVEC and seal the barrier. This astrocyte-BMVEC coculture model has many characteristics of the human BBB, including expression of GLUT-1, high transendothelial electrical resistance, and impermeability to albumin and ^3H inulin [101, 111].

In other studies we showed that HIV infected $CD14^+CD16^+$ monocytes transmigrated in greater numbers than did their uninfected counterparts across the BBB in response to CCL2 [10, 25]. The increase in transmigration of HIV infected cells did not occur in the absence of the chemokine, indicating that this process is dependent upon CCL2. Additionally, high numbers of $CD14^+CD16^+$ cells crossed the BBB even at very low levels of HIV infection. We demonstrated that this was due to the significantly heightened sensitivity of HIV infected $CD14^+CD16^+$ monocytes to CCL2 and was mediated, at least in part, by increased CCR2[25]. These findings indicate that even with successful antiretroviral therapy, HIV infected $CD14^+CD16^+$ monocytes may continually transmigrate into the CNS of infected individuals promoting inflammation and infection of the brain.

Using cells matured in our *in vitro* culture method, we determined that $CD14^+CD16^+$ monocytes expressed high amounts of the junctional proteins JAM-A, ALCAM, CD99, and PECAM-1 which we hypothesized would facilitate their preferential transmigration across the BBB[25]. We showed that two of these proteins, JAM-A and ALCAM, were critical for the transmigration of both HIV infected and uninfected monocytes across the BBB model in response to CCL2, as blocking antibodies significantly inhibited the number of transmigrating cells[25]. These data suggest that targeting junctional proteins that enable transmigration of infected cells across the BBB should be examined as a therapeutic approach to limit neuroinflammation.

CCL2 may also facilitate monocyte transmigration by dysregulating the cell-cell junctions and tight junctions that occur between BMVEC[125-128]. These points of BMVEC contact are critical for impermeability of the barrier. The tight junction protein, JAM-A, is localized at endothelial cell junctions under baseline conditions to maintain vasculature integrity. CCL2 results in redistribution of JAM-A to the apical surface of the BMVEC [129]. This altered localization would compromise the homotypic JAM-A interactions that occur

between opposing endothelial cells, potentially contributing to increased paracellular entry of monocytes into the brain. The apical expression of JAM-A may also contribute to monocyte transmigration into the brain by facilitating monocyte-endothelial cell JAM-A interactions. We showed that treatment of BMVEC with CCL2 caused a transient decrease in endothelial cell junctions which resulted in a weakened structural integrity of the BBB [130]. In this study, CCL2 treatment of BMVEC resulted in an increase in surface PECAM-1 which may also facilitate monocyte transmigration across the BBB[130].

BBB compromise may also occur as a result of viral proteins[131-135], HIV infection of astrocytes[60, 136, 137], increased CCL2 production [87, 88, 138], and dysregulation of endothelial cell activation/inflammatory markers[139]. These represent additional mechanisms that may facilitate monocyte entry into the CNS of HIV infected individuals.

Monocytes as a Viral Reservoir

Despite the success of antiretroviral therapy, monocytes and macrophages remain a viral reservoir, both peripherally and within the brain[140, 141], and represent a major challenge in HIV neuropathogenesis. Latently infected monocytes persist even in aviremic individuals[142]. The half life of viral DNA within these cells is twice that of both resting and activated CD4 T cells[143]. As monocytes are only present in the periphery for 1-3 days, the presence of viral DNA within these cells indicates their recent, low-level infection even when plasma viral loads remain undetectable. $CD14^+CD16^+$cells have been shown to contain more viral DNA as compared to monocytes that are $CD14^+CD16^-$[144].

Viral DNA within these circulating $CD14^+CD16^+$ monocytes may be predictive of cognitive decline in HIV infected individuals [145], further indicating the importance of this mature monocyte population in the pathogenesis of HAND. Integrated proviral DNA within these cells persists, despite cART, and may be a risk factor for the development of HIV associated cognitive deficits. HIV proviral DNA was quantified in PBMC isolated from infected individuals with normal cognition and from those with HAD. In both cART-treated and cART-naïve people, there was a significantly higher amount of proviral DNA within the PBMC from impaired individuals as compared to those with normal cognition [145, 146]. $CD14^+CD16^+$ monocytes were the primary source of this proviral DNA, suggesting that the infection of these cells promotes cognitive impairment [145, 147]. In another study, viral DNA was significantly associated with deficits in 8 out of 9 domains frequently impaired in HAND, including verbal memory, executive function, and motor skills and motor speed [148]. HIV DNA within $CD14^+CD16^+$ monocytes was correlated with cognitive impairment both prior to and nearly one year after cART treatment [38]. There was no association between HIV RNA or plasma viral load and cognitive impairment [38]. Ultimately, failure to clear viral DNA from these $CD14^+CD16^+$ cells was associated with persistent cognitive impairment, even after many years of successful cART [147, 149]. These studies further underscore the importance of this $CD14^+CD16^+$monocyte population as a viral reservoir that contributes to the neuropathogenesis of HIV.

It is difficult to determine the extent to which latently infected monocytes/macrophages will produce infectious virus in the brain. Infectious, replication competent virus can be found within circulating monocytes from cART treated individuals who have achieved viral

suppression [30, 31, 33, 34, 140, 143, 150, 151] indicating that, to some degree, monocytes continue to remain a source of viral production despite successful antiretroviral therapy. Regimens that better target cells of the monocyte lineage are important to eradicating both peripheral and CNS viral reservoirs, as they are the major source of virus production in the brain. A scale has been developed to assess the efficacy of antiretroviral regimens to inhibit HIV infection of monocytes/macrophages[152]. cART regimens with higher monocyte efficacy scores more effectively inhibited the *in vitro* infection of monocytes and macrophages as compared to treatments with lower scores. HIV seropositive people receiving antiretroviral regimens with lower monocyte efficacy scores had a significantly higher likelihood of cognitive impairment[152]. This occurred even in individuals who had undetectable plasma viral loads. These findings indicate that therapeutic strategies that specifically target these cells may be critical to decreasing the prevalence of HAND.

Monocytes and Macrophages as Biomarkers of HAND

The cognitive deficits associated with HIV infection are multifactorial and dynamic. Easily accessible, reliable markers that reflect the complexity of neuroAIDS are necessary to understand more completely the mechanisms that contribute to its pathogenesis. Additionally, monocyte/macrophage cell surface and activation markers may be useful tools to monitor the progression of HAND and to assess CNS dysregulation. Some of these markers are CD163, osteopontin, neopterin and the cellular isoform of the human prion protein (PrP^{C}).

CD163 is a scavenger cysteine-rich family member that is specifically expressed on cells of the monocyte lineage[153], including microglia, perivascular macrophages, and peripheral blood monocytes. CD163 is highly expressed in the brain during HIV and SIV encephalitis [76, 154, 155]. During chronic SIV infection, CD163 expressing microglia may also be a marker of BBB compromise [156].

$CD14^{+}CD16^{+}CD163^{+}$ peripheral blood monocytes repopulate the perivascular macrophage population within the CNS of SIV infected macaques [155]. CD163 is highly expressed on human $CD14^{+}CD16^{+}$ monocytes, as compared to cells without surface CD16, during baseline conditions [157, 158] and in inflammation [159]. These monocytes are present in greater numbers in seropositive individuals with detectable viral loads and may be reflective of disease progression [160].

In addition to being cell associated, CD163 is shed from the monocyte cell surface and accumulates in the plasma[161, 162]. The chronic immune activation that occurs as a result of HIV infection may, in part, mediate increased shedding of CD163 [163, 164]. The amount of this soluble form (sCD163) in the plasma of people with HIV is similar to that present in significantly older, seronegative individuals [164]. sCD163 is increased in virally suppressed seropositive people with cognitive impairment, indicating that monocyte activation persists in individuals with HAND despite effective cART [165]. Circulating sCD163 is elevated even in those who have mild cognitive deficits and was shown to be predictive of impairment over time[165].

Osteopontin is a secreted glycoprotein implicated in HIV neuropathogenesis that is present as a component of the extracellular matrix and as a soluble cytokine. Although $CD14^+CD16^-$monocytes do not express osteopontin, it is increased during *in vitro* maturation [11] and upon macrophage differentiation [166]. Osteopontin is associated with many inflammatory disorders [167] and is induced by *in vitro* treatment with cytokines associated with HIV CNS disease, including IL-6 and IL-1β [168].

Osteopontin is increased in the brain of SIV encephalitic monkeys [169] and in HIV infected individuals with cognitive impairment [170, 171]. In a transfected epithelial carcinoma cell line, osteopontin increased CD163 and CCL2[172], suggesting that its expression in the brain during HIV infection may facilitate monocyte entry into the CNS. The presence of osteopontin added to the basolateral side of an *in vitro* BBB model decreased the reverse transmigration of $CD14^+CD16^+$, but not $CD14^+CD16^-$, monocytes indicating that it may contribute to the neuropathogenesis of HAND by selectively retaining the cell population that is highly susceptible to HIV infection and that acts as a viral reservoir[173].

Osteopontin may also mediate HIV infection of $CD14^+CD16^+$ monocytes and resident CNS cells. Genetic knockdown of osteopontin during infection of MDM caused a significant decrease in HIV replication [171]. Additionally, osteopontin may promote the survival and contribute to the presence of CNS viral reservoirs as it has been shown to protect monocytes and macrophages from apoptosis [168, 173].

Osteopontin is increased in the plasma and CSF of monkeys with SIV encephalitis and in HIV infected individuals[170, 171]. Interestingly, plasma osteopontin was increased only in individuals with cognitive impairment. A longitudinal analysis of monkeys infected with SIV demonstrated that plasma osteopontin increased before the development of CNS abnormalities [170]. Circulating osteopontin, which remains increased despite antiretroviral therapy[174], may be another useful peripheral blood marker to monitor the development of HAND.

Two additional biomarkers of HIV associated cognitive impairment may include neopterin, a marker of monocyte/macrophage activation[108, 165, 175, 176] and PrP^C, which is expressed on monocytes and many resident CNS cells[177-180]. PrP^C is implicated in monocyte mediating diapedesis [181]. Our laboratory found soluble PrP^C is increased in the CNS of those with HAND, but not in HIV infected individuals without cognitive impairment [182]. Further discussion about the role of PrP^C as a biomarker of HAND can be found in a recent review [183].

Concluding Remarks

Monocyte transmigration into the CNS of HIV infected individuals establishes the CNS as a viral reservoir, promotes neuroinflammation, and significantly contributes to the pathogenesis of HAND, which continues to occur despite successful cART. Chronic neuroinflammation is believed to be ongoing in cART-treated, HIV infected people and is attributed, in part, to the entry of mature $CD14^+CD16^+$ monocytes into the brain parenchyma. This cell population is increased in the peripheral blood of HIV infected individuals and, due to CNS chemotactic signals including CCL2 and CXCL12,

transmigrates across the BBB, enters the CNS, and initiates the inflammatory cascade resulting in cognitive impairment. Identification of therapeutic interventions that target this monocyte viral reservoir and decrease their transmigration across the BBB, and the resulting neuroinflammation, are critical to preventing the neuropathogenesis of HIV.

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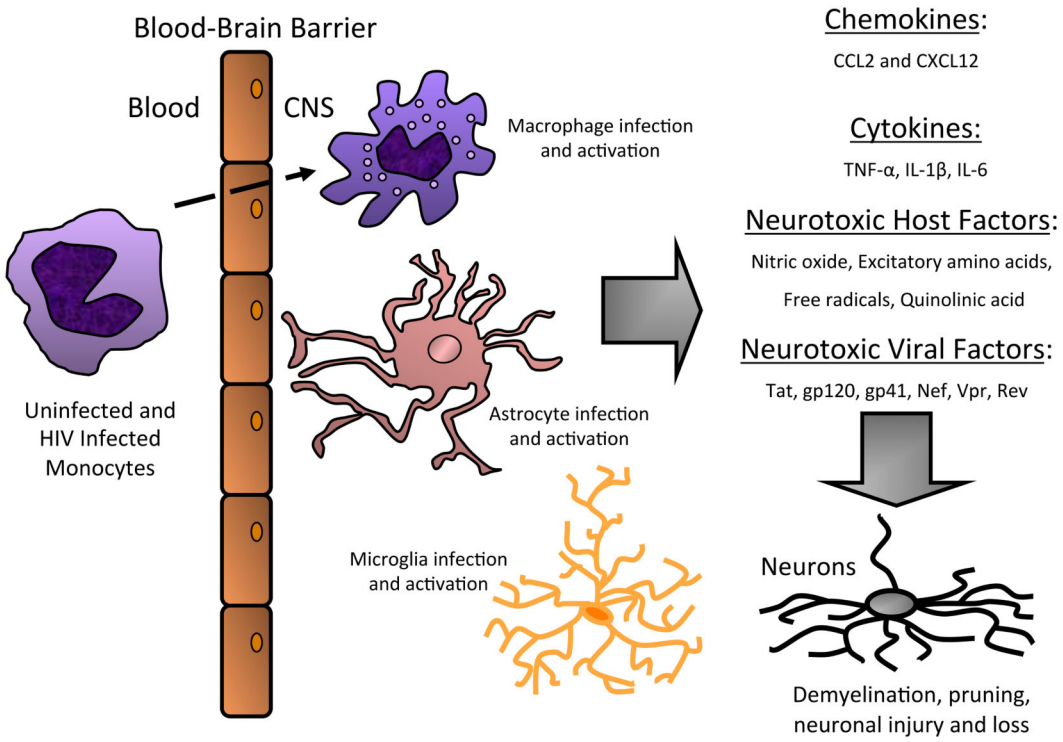

Figure 1. Schematic Representation of Mechanisms of CNS HIV Infection and Damage

$CD14^{+}CD16^{+}$ monocytes, which were infected with HIV while in the peripheral circulation, transmigrate across the BBB and enter the brain parenchyma in response to baseline levels of chemokine. Infectious virus is released as the mature monocytes enter the CNS and also as they differentiates into macrophages. This virus can then infect macrophages, microglia, and a small percent of astrocytes. Viral proteins, such as tat and gp120, and inflammatory mediators are released from these infected cells which activate the surrounding resident CNS cells. The activated cells produce cytokines, including TNF-α, IL-1β, and IL-6 and chemokines, including CCL2 and CXCL12. The elevated levels of CCL2 and CXCL12 promote additional influx of uninfected and HIV infected monocytes into the brain. Small molecules, such as arachidonic acid and quinolinic acid, are also produced. The elaboration of chemotactic, inflammatory, and neurotoxic factors perpetuates the cycle of chronic inflammation within the CNS and ultimately result in the neuronal damage and loss associated with HAND.

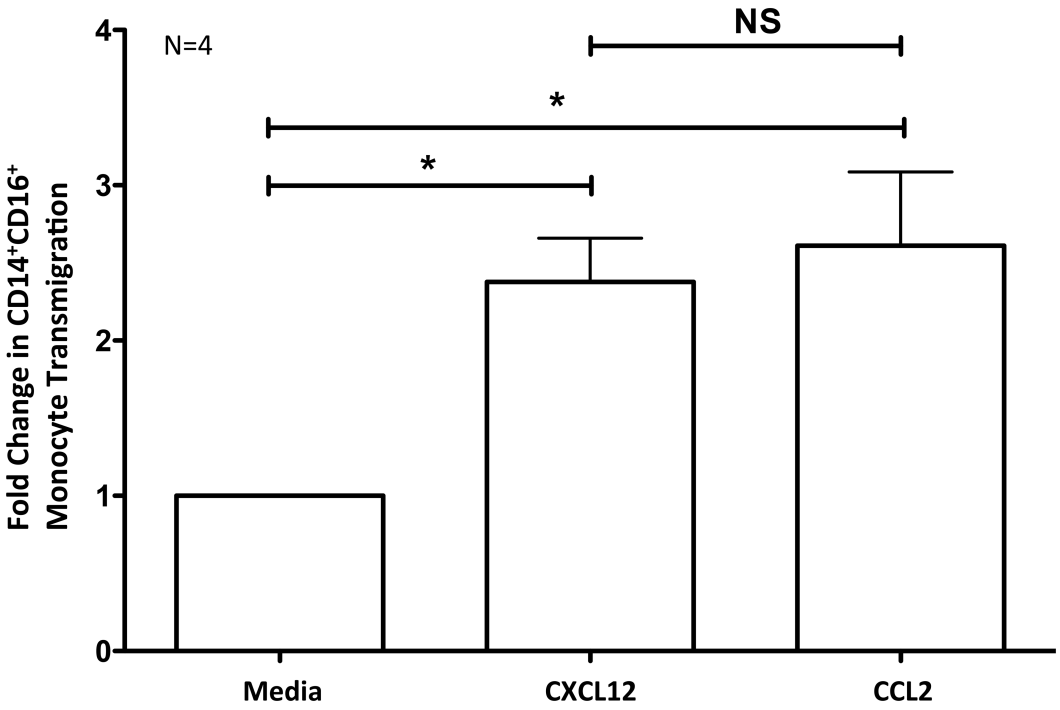

Figure 2. CXCL12 and CCL2 Promote Transmigration of $CD14^+CD16^+$ Monocytes Across a Tissue Culture Model of the Human BBB

Monocytes from 4 independent donors were cultured nonadherently for 3 days in our system and then added to the top of our *in vitro* model of the human BBB and transmigrated in response to media alone, 25 ng/mL CXCL12, and 200 ng/mL CCL2 for 24 hours. Both chemokines promoted increased transmigration of $CD14^+CD16^+$ monocytes across the BBB as compared to media alone (*$p<0.05$, $n=4$). NS indicates no significant change.