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Review

Monocyte/macrophage trafficking in acquired immunodeficiency syndrome encephalitis: Lessons from human and nonhuman primate studies

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Here the authors discuss evidence in human and animal models supporting two opposing views regarding the pathogenesis of human immunodeficiency virus (HIV) in the central nervous system (CNS): (1) HIV infection in the CNS is a compartmentalized infection, with the virus-infected macrophages entering the CNS early, infecting resident microglia and astrocytes, and achieving a state of latency with evolution toward a fulminant CNS infection late in the course of disease; or alternatively, (2) events in the periphery lead to altered monocyte/macrophage (MΦ) homeostasis, with increased CNS invasion of infected and/or uninfected MΦs. Here the authors have reevaluated evidence presented in the favor of the latter model, with a discussion of phenotypic characteristics distinguishing normal resident microglia with those accumulating in HIV encephalitis (HIVE). CD163 is normally expressed by perivascular MΦs but not resident microglia in normal CNS of humans and rhesus macaques. In agreement with other studies, the authors demonstrate expression of CD163 by brain MΦs in HIVE and simian immunodeficiency virus encephalitis (SIVE). CNS tissues from HIV-sero positive individuals with HIVE or HIV-associated progressive multifocal leukoencephalopathy (PML) were also examined. In HIVE, the authors further demonstrate colocalization of CD163 and CD16 (FcγIII recptor) gene expression, the latter marker associated with HIV infection of monocyte *in vivo* and permissivity of infection. Indeed, CD163$^+$ MΦs and microglia are often productively infected in HIVE CNS. In SIV infected rhesus macaques, CD163$^+$ cells accumulate perivascularly, within nodular lesions and the parenchyma in animals with encephalitis. Likewise, parenchymal microglia and perivascular MΦs are CD163$^+$ in HIVE. In contrast to HIVE, CD163$^+$perivascular and parenchymal MΦs in HIV-associated PML were only associated with areas of demyelinating lesions. Interestingly, SIV-infected rhesus macaques whose viral burden was predominantly at 1×10^6 copies/ml or greater developed encephalitis. To further investigate the relationship between CD163$^+$/CD16$^+$ MΦs/microglia in the CNS and altered homeostasis in the periphery, the authors performed flow-cytometric analyses of peripheral blood mononuclear cells (PBMCs) from SIV-infected rhesus macaques. The results demonstrate an increase in the percent frequency of CD163$^+$/CD16$^+$ monocytes in animals with detectable virus that correlated significantly with increased viral burden and CD4$^+$ T-cell decline. These results suggest the importance of this monocyte subset in HIV/SIV CNS disease, and also in the immune pathogenesis of lentiviral infection. The authors further discuss the potential role of CD163$^+$/CD16$^+$ monocyte/MΦ subset expansion, altered myeloid homeostasis, and potential consequences for immune polarization and suppression. The results and discussion here suggest new avenues for the development of acquired immunodeficiency syndrome (AIDS) therapeutics and vaccine design. *Journal of NeuroVirology* (2008) **14,** 318–326.

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Dedicated to the memory of Opendra "Bill" Narayan.

Human immunodeficiency virus type 1 (HIV-1)–associated dementia (HIV-D) is a syndrome of motor and cognitive dysfunction observed in approximately 5% to 10% of patients infected with HIV-1 with acquired immune deficiency syndrome (AIDS) (McArthur *et al*, 1993; Sacktor *et al*, 2001). The neuropathogenesis of HIV-D is not completely understood. HIV enters the central nervous system (CNS) early, during the acute phase of infection; however, the action of cytotoxic T cells eliminates productively infected cells. Later during the course of disease, productive infection of the CNS ensues, with the concomitant development of CNS disease. The source and mechanism of this latter infection of the CNS has been a matter of considerable debate. It has been unclear if virus recrudesces from a latent reservoir or, alternatively, there is a new invasion of virus-infected cells. In a previous review (Fischer-Smith and Rappaport, 2005), we discussed two models: the Trojan Horse Model and the Late Invasion Model. In the Trojan Horse Model, the virus enters the CNS early, and replicates at low levels as a reservoir separated from the periphery. A viral phenotype that is more virulent in the context of the CNS emerges, leading to the development of disease. In the Late Invasion Model, uncontrolled virus replication and resulting immune deficiency lead to alterations in the myeloid differentiation pathway, promoting the expansion of an activated monocyte subset that is capable of tissue invasion.

In support of the Trojan Horse Model, several studies suggest compartmentalization of HIV in the CNS through comparisons of viral quasispecies inside and outside the CNS compartment (Clements *et al*, 2002, 2005). Many of these studies have compared plasma virus with virus found in cerebrospinal fluid (CSF) (Cunningham *et al*, 2000; Stingele *et al*, 2001; Strain *et al*, 2005; Tashima *et al*, 2002). Such comparisons may reflect the differences in the cellular source of virus, rather than viral evolution *per se*, because plasma virus is derived from lymphoid tissues, involving both infected T cells and macrophages (MΦs), and CSF virus is derived from infected perivascular MΦs and microglia. Studies in simian immunodeficiency virus (SIV)-infected rhesus macaques model also provide some evidence in support of a compartmentalized infection. These studies demonstrate suppression of virus replication in the CNS as early as 21 days, without loss of CNS viral DNA, with virus replication recrudescing later during the course of disease in a rapid model of SIV encephalitis (SIVE) (Barber *et al*, 2004). MΦ invasion does indeed occur in this model, however, suggesting that microglial activation is not the sole contributor to CNS disease. MΦ invasion is not limited to the CNS in this model, and peripheral neuropathy is also a consequence of global MΦ activation and trafficking (Laast *et al*, 2007). Although these studies do not preclude the contribution of invading MΦs, the constant level of viral DNA in CNS suggested a major contribution of a latent CNS reservoir.

There are several lines of evidence, however, that support an alternative hypothesis, as depicted in the Late Invasion Model. It has been proposed that virus entry into the CNS is due largely to the trafficking of HIV-1–infected monocytes/MΦs from circulation (Meltzer *et al*, 1990). Accordingly, the number of total brain MΦs is dramatically increased in HIV encephalopathy (HIVE), the pathology of HIV-D, without additional evidence of local proliferation by these cells (Fischer-Smith *et al*, 2004; Glass *et al*, 1995). Furthermore, MΦs/microglia represent the principle productive reservoir of HIV1 infection in the CNS (Kure *et al*, 1991; Porwit *et al*, 1989; Pumarola-Sune *et al*, 1987; Rostad *et al*, 1987). The contribution of infected MΦs and microglial cells to neuronal injury through secretion of viral and host factors has been the subject of numerous reviews (Fischer-Smith and Rappaport, 2005; Gonzalez-Scarano and Martin-Garcia, 2005; Kaul *et al*, 2001).

The Late Invasion Model assumes that there is a great influence of HIV/SIV infection status within the peripheral compartment that contributes to the development of CNS disease. Previous studies comparing HIV-1 Gp120 sequences have demonstrated the greatest similarity between envelope sequences derived from brain with those derived from bone marrow and blood (Liu *et al*, 2000). The role of monocyte/MΦ trafficking from the periphery into the CNS is further supported by the beneficial effects of highly active antiretroviral therapy (HAART) despite poor CNS penetrance of most antiretroviral compounds (Vehmas *et al*, 2004).

Determining the origin of the significant number of brain MΦs in CNS with or without disease has been difficult because no single cluster of differentiation (CD) can conclusively discriminate between resident microglia and perivascular MΦs. As such, combined CD markers have been used to make this distinction. Perivascular MΦs are positive for CD14 (lipopolysaccharide [LPS] receptor) and CD45 (leukocyte common antigen [LCA]); however, microglia do not express detectable levels of these antigens by standard immunohistochemical methods (Ford *et al*, 1995; Sedgwick *et al*, 1993; Ulvestad *et al*, 1994; Williams *et al*, 1992). Previously, we identified two populations of activated MΦs in the CNS of patients with HIVE (Fischer-Smith *et al*, 2001). MΦs accumulating perivascularly are $CD14^+/CD45(LCA)^+/CD16^+$ ($Fc\gamma III$ receptor), and appear to be the principal reservoir of productive HIV-1 infection in the CNS. Similar observations were also reported in SIVE (Williams *et al*, 2001). These cells are phenotypically similar to a subpopulation of monocytes reported to be expanded in patients with HIV-D, as compared to patients with HIV-1 infection without dementia and seronegative controls (Pulliam *et al*, 1997). Importantly, $CD16^+$ monocytes preferentially harbor HIV *in vivo*, are more permissive to HIV

infection than $CD16^-$ monocytes, and are likely important as reservoirs of infection and tissue dissemination (Ellery *et al*, 2007; Joworoski *et al*, 2007). Additionally, ramified cells with microglial morphology located within the brain parenchyma were also found to harbor productive HIV-1 infection, although to a lesser degree than MΦs located perivascularly (Fischer-Smith *et al*, 2001). These ramified cells are $CD14^-$/$CD45(LCA)^-$/$CD16^+$ (Fischer-Smith *et al*, 2001). We previously suggested that based on their increased numbers and immunophenotype, these cells may represent activated resident microglia and/or MΦs that have recently migrated from the peripheral blood into the brain parenchyma, with apparent loss of CD14 and CD45(LCA) expression (Fischer-Smith *et al*, 2001). In support of the latter hypothesis, the increase in total brain MΦs associated with HIVE appears to be due to trafficking of monocytes/MΦs into the CNS from the periphery, rather than local microglial proliferation (Fischer-Smith *et al*, 2004).

In SIVE and HIVE, MΦs/microglia represent the only cells in the CNS to demonstrate productive SIV or HIV-1 infection, respectively. Microglia, the resident MΦ of the brain, populate the CNS during fetal development and for a brief period after birth, after which there is little or very slow turnover (Unger *et al*, 1993). In contrast, perivascular MΦs are more rapidly and continuously replaced by monocytes from circulation (Hickey and Kimura, 1988). CD163, a monocyte/MΦ-specific scavenger receptor for hemoglobin-haptoglobin complex (Kristiansen *et al*, 2001), is reported to be expressed by perivascular MΦs, but not resident microglia, in normal human CNS (Fabriek *et al*, 2005b; Rezaie and Male, 2003). CD163 expression has been identified previously in HIVE and SIVE CNS, but not in AIDS patients without CNS disease with little, and no expression was observed in variant Creutzfeldt-Jakob disease or Alzheimer's dementia (Roberts *et al*, 2004). From these studies, the authors suggested that the CD163 expression seen in HIVE and SIVE represents a specific type of microglial activation seen only in some pathogen-induced inflammatory CNS conditions (Roberts *et al*, 2004).

In view of the disparity in the expression pattern of CD163 by perivascular MΦs versus microglia that has been previously reported (Fabriek *et al*, 2005a), together with the increases in total MΦs and microglia we previously reported in HIVE (Fischer-Smith *et al*), we sought to revisit this issue with the notion that the accumulation of $CD163^+$ "microglia" in HIVE and SIVE occur as a consequence of monocyte/MΦ trafficking and the adaptation and engraftment of peripheral derived MΦs within the brain parenchyma.

In agreement with previous reports (Kim *et al*, 2006; Roberts *et al*, 2004), we observed significant CD163 accumulation within the perivascular cuff and nodular lesions in HIVE CNS (Figure 1A, panels F and G). Additionally, abundant CD163 expression was seen in cells in the brain parenchyma, many having ramified microglial morphology (Figure 1A, panel C). In consideration of other reports that demonstrate CD163 expression by perivascular MΦs but not resident microglia in normal CNS (Fabriek *et al*, 2005b; Rezaie and Male, 2003), CD163 expression by parenchymal microglia is in agreement with our previously reported findings suggesting that the large numbers of ramified microglia accumulating in HIVE are peripheral blood derived (Fischer-Smith *et al*, 2004; Fischer-Smith *et al*, 2001). Furthermore, the proposed "activation" of CD163 expression in microglia in HIVE would not explain the increase in the numbers of MΦs/microglia we observed in HIVE CNS (Fischer-Smith *et al*, 2004), without evidence for proliferation. In contrast to HIVE, brain tissues derived from seronegative and HIV-infected individuals exhibit few $CD163^+$ perivascular cells and the absence of detectable $CD163^+$ parenchymal cells (Figure 1A, panels A, B, D, and E).

As we had observed and reported previously, $CD16^+$ cells are seen in the parenchyma and perivascular cuffs of HIVE CNS (Figure 1B, panels A and D). Colocalization studies revealed that the majority of these $CD16^+$ cells also expressed the perivascular MΦ marker, CD163 (Figure 1B, panels C and F). $CD16^+$ monocytes may be important to the development of HIV-D and HIVE where the expansion of this subset was demonstrated in patients with AIDS/dementia over AIDS patients without dementia and seronegative individuals (Pulliam *et al*, 1997). $CD16^+$ monocytes exhibit features of tissue MΦs and are more phagocytic and express high levels of inflammatory cytokines (Scherberich and Nockher, 2000). It was suggested that these cells are more invasive and thus able to enter the CNS compartment in HIV-D (Pulliam *et al*, 1997). In support of this hypothesis, we reported a significant accumulation of $CD16^+$ cells in the CNS of patients with HIVE (Fischer-Smith *et al*, 2001). In our current study, we find CD163 colocalizes with CD16 to a significant degree throughout the CNS in HIVE. Enhanced CD163 expression on $CD16^+$ monocytes may contribute to the invasiveness of these cells, as CD163 has been demonstrated to augment monocyte adherence to LPS or cytokine-stimulated endothelial cells (Wenzel *et al*, 1996). Interestingly, the $CD16^+$ monocyte subset reportedly shows the highest CD163 expression of all human monocyte subsets (Buechler *et al*, 2000).

Additionally in HIVE CNS, many $CD163^+$ cells were found to harbor productive infection, as indicated by colocalization of CD163 and HIV-1 p24. The majority of these cells were found within the perivascular space and in nodular lesions (Figure 1C, panels F and I). HIV-1 p24 positivity was also observed in a number of $CD163^+$ cells located in the brain parenchyma; however, some $CD163^+$ cells do not appear to harbor productive infection (Figure 1C, panel C).

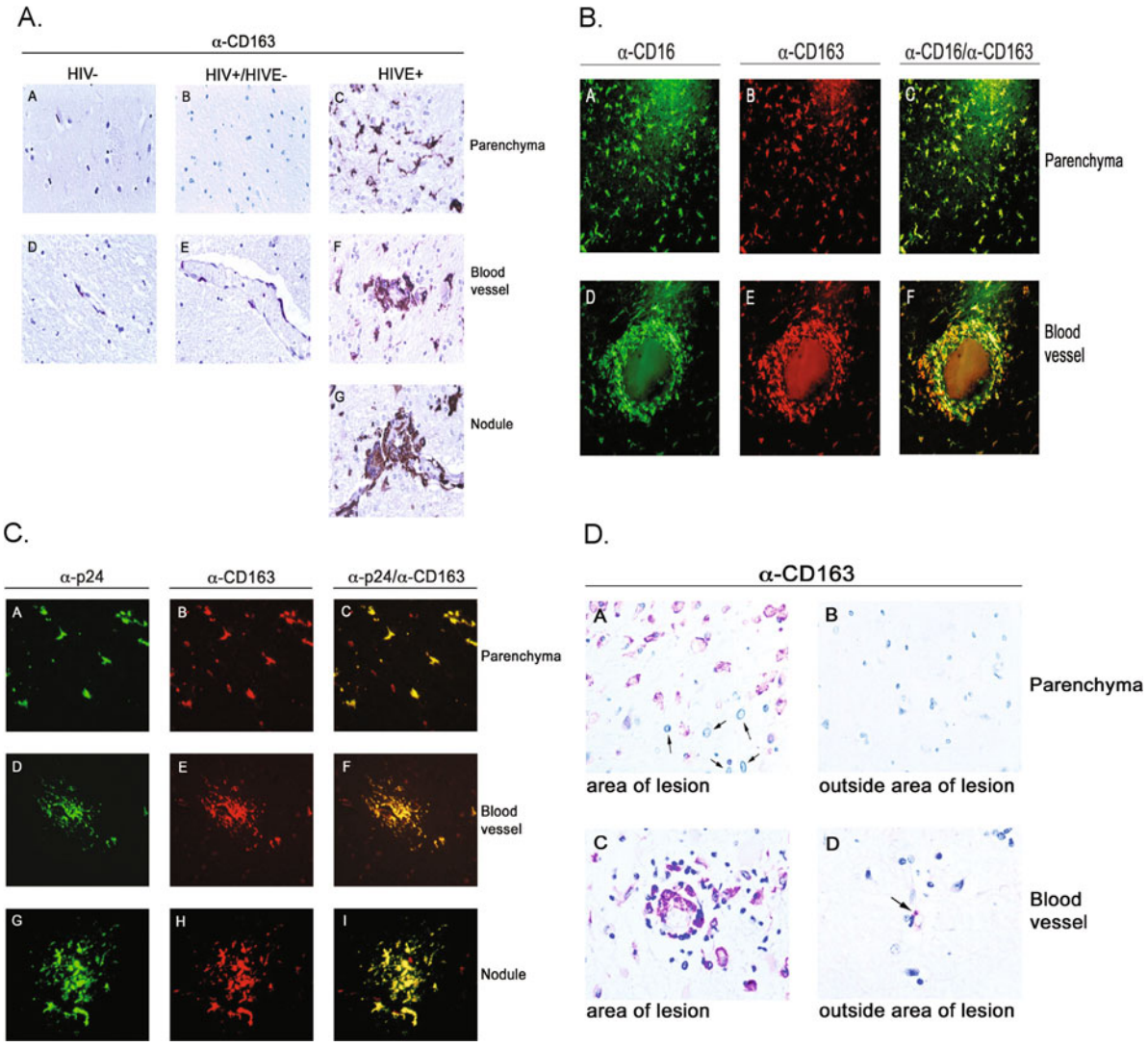


Figure 1 HIVE CNS shows significant accumulation of CD163$^+$ cells that colocalizes with CD16 and harbors productive HIV-1 infection. All panels are shown at 40× magnification. A significant number of CD163$^+$ MPs are observed in HIVE when compared to HIV-1–infected individuals without dementia and seronegative controls (**A**). Double-immunofluorescence studies of HIVE CNS tissue using sequential application of antibodies against human CD163 and CD16 on the same tissue section shows that virtually all CD163$^+$ cells colocalize with CD16 (**B**). Similar analyses on HIVE CNS tissue using antibodies against CD163 and HIV-1 p24 revealed HIV-1 p24$^+$ cells in patchy areas of the brain parenchyma, around blood vessels, and within nodular lesions (**C**). The predominance of these cells located perivascularly and within nodular lesions were found to be CD163$^+$. Additionally, patches of ramified CD163$^+$ cells in the parenchyma appear to harbor productive infection. PML CNS tissue reveals a different pattern of CD163 positivity from that seen in HIVE. (**D**) Panels A and B illustrate brain parenchyma. Panels C and D show blood vessels. Panels A and C demonstrate a PML lesion. Panels B and D represent areas outside of the PML lesions. A significant number of CD163$^+$ MPs are observed within PML lesions. These cells are seen both in the parenchyma and the perivascular space. Interestingly, CD163$^+$ cells with ramified microglial morphology are not observed in PML, as is seen in HIVE, but have a round, foamy morphology. Outside of PML lesions, only limited CD163$^+$ cells are observed perivascularly. Parenchymal CD163$^+$ cells are not observed outside of lesions.

To gain additional understanding into the presence of the considerable number of CD163$^+$ cells in the CNS of patients with HIVE, we investigated brain tissue from patients with HIV-1 infection/AIDS with progressive multifocal leukoencephalopathy (PML) for CD163 expression. PML is a demyelinating disorder of the CNS associated with the polyomavirus, JC virus. In the CNS, JC virus infects and replicates in oligodendrocytes, ultimately destroying the infected cell, resulting in regions of myelin loss (lesions) in multiple areas of the brain (for review, see Wyen *et al*, 2005). In our studies, we found significant CD163 positivity by perivascular MΦs, as well as MΦs/microglia located in the brain parenchyma; however, these CD163$^+$ cells were primarily localized to within the area of the PML lesion(s) (Figure 1D, panels A and C). Additionally, parenchymal CD163$^+$ cells do not demonstrate the microglial morphology we see in HIVE (Figure 1A, panel C), but had a round and foamy appearance (Figure 1D,

panel A). In areas outside of PML lesions, CD163$^+$ cells were not seen in the parenchyma (Figure 1D, panel B) and only few cells were observed perivascularly (Figure 1D, panel D). This is in stark contrast to what was observed in HIVE CNS tissues, which demonstrated a generalized CD163 positivity throughout the white matter in tissues associated with frontal cortex and basal ganglia (Figure 1A, panels C, F, and G). Presumably, the CD163$^+$ foamy MΦs seen in PML originated as monocytes, which were recruited into the CNS to areas of involvement. They become lipid-laden by clearing up myelin released from the infected oligodendrocytes, resulting in their foamy appearance. In support of this hypothesis, CSF from PML patients has demonstrated a significantly higher concentration of monocyte chemoattractant protein-1 (MCP-1), a chemokine involved in chemotactic migration of monocytes, when compared to HIV-1–infected individuals without PML and seronegative controls (Marzocchetti *et al*, 2005). Interestingly, CD163 expression by foamy MΦs has also been observed in multiple sclerosis (MS) (Fabriek *et al*, 2005b), a demyelinating CNS disease also involving monocyte recruitment. Also in MS, MCP-1 expression has been observed in astrocytes and MΦs within acute lesions (Simpson *et al*, 1998).

In addition to our studies in human tissues, we expanded our studies to include examination of uninfected and SIV_{mac251}-infected rhesus macaques with and without CNS complications for alterations in CD163$^+$ peripheral blood monocyte subsets by flow cytometry and assessed how these changes might correlate with the development of CNS disease. These studies were performed in eight SIV_{mac251}-infected animals during the natural course of SIV disease. An additional two uninfected animals and eight SIV_{mac251}-infected animals treated with antiretroviral therapy (ART) (PMPA and FTC, with varying degrees of success) were included in the blood monocyte studies.

Of the eight SIV-infected animals that were followed for up to 1 year without treatment, four developed SIVE as determined by immunohistochemical examination. *In situ* hybridization using an RNA probe against SIV_{mac239} identified productively infected cells in animals with encephalopathy (Figure 2A, panels C, F, and G) but not in SIV-infected animals without CNS disease (Figure 2A, panels B and E). Similar to that seen in HIVE, the majority of SIV+ cells form perivascular cuffs and nodules (Figure 2A, panels F and G) with few positive cells seen in the parenchyma (Figure 2A, panel C). Our immunohistochemical studies would suggest that these cells are also largely CD163$^+$ (Figure 2B, panels F and G) and may support the hypothesis that the virus seen in the CNS in SIVE has recently entered the CNS compartment from the peripheral blood.

In view of the observation that CD163 is not normally expressed by resident microglia in normal brain tissue (Fabriek *et al*, 2005a), the numerous ramified CD163$^+$cells we observe in SIVE and HIVE suggest a mechanism involving emigration of MΦs into the CNS compartment from the peripheral blood and adaptation to microglial morphology. We have not excluded, however, the possibility that CD163 expression in microglia represents an unusual state of microglia activation. Indeed, CD163 expression can be induced by the

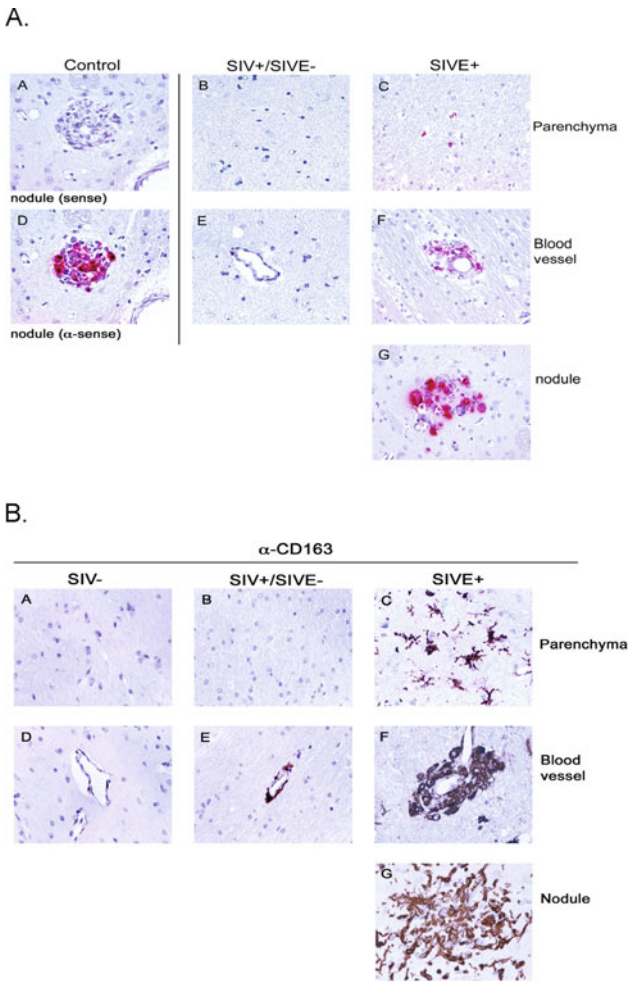


Figure 2 CD163$^+$ cell accumulation and productive SIV infection in the CNS of rhesus macaques. *In situ* hybridization using α-sense RNA probe against SIVmac239 reveals productive SIV infection confined primarily to cells located perivascularly and within nodules in macaques with encephalopathy (**A**). Separate immunohistochemistry studies revealed that these cells are also CD163$^+$ (**B**). Rare positive cells are also observed within the brain parenchyma (**A**). Productive infection is not seen in SIV-infected animals without encephalopathy. (**A**) Panels A and D demonstrate the specificity of the probe used where panel A shows a nodular lesion from a section of tissue treated with a sense probe and panel D shows the same nodule from tissue treated with the α-sense probe. (**B**) Infrequent CD163$^+$ perivascular MΦs are observed in seronegative CNS tissue, with slightly more seen in SIV-infected animals without encephalopathy. SIVE, however, shows significant accumulation of CD163$^+$ MΦs located perivascularly and within nodular lesions. Numerous CD163$^+$ ramified microglia are also observed in the brain parenchyma.

anti-inflammatory mediators, glucocorticoids and interleukin (IL)-10, as well as by the proinflammatory cytokine, IL-6 (Buechler *et al*, 2000; Hogger *et al*, 1998; Sulahian *et al*, 2000). Additionally, peripheral blood monocytes differentiated to MΦs in the presence of macrophage colony-stimulating factor (M-CSF) have shown increased CD163 mRNA and protein expression (Buechler *et al*, 2000). M-CSF activation by HIV infection in MΦs (Gruber *et al*, 1995) may promote alterations in monocyte/MΦ homeostasis. Indeed, M-CSF levels in plasma and CSF correlate inversely with time to death in patients with advanced HIV disease (Sevigny *et al*, 2007).

To begin to investigate the relationship between alterations in circulating monocyte subsets and the development of CNS disease, we performed flow cytometric studies on isolated peripheral blood mononuclear cells (PBMCs) in SIV_{mac251}-infected rhesus macaques during the chronic phase of SIV infection. These studies were performed to investigate potential alterations in $CD163^+$ monocyte subsets and associated changes in viral burden. This analysis included PBMCs from untreated animals, as well as PBMCs from additional SIV_{mac251}-infected animals treated with antiretroviral therapy (ART). Data were collected at days 154, 168, and 196 post challenge. Monocytes were identified first by forward and side scatter parameters, then by CD14 expression. In our studies we found that, like humans, rhesus macaques express CD163 by the majority of monocytes with a subset of these expressing CD16. This $CD163^+/CD16^+$ monocyte ($CD14^+$) subset was expanded in all chronically infected animals with detectable virus as compared to those with undetectable viral loads and seronegative animals, and this expansion was found to correlate with a greater viral load (Figure 3, panel A). Interestingly, animals with CNS disease at necropsy generally maintained very high viral loads ($>1 \times 10^6$ copies/ml) throughout infection (Graph 1). Additionally, the expansion of this monocyte subset was also found to correlate inversely with the number of $CD4^+$ T cells in animals with counts below 1100 cells/μl (Figure 3, panel B). These data suggest that $CD14^+/CD163^+/CD16^+$ monocytes may play a role in virus production and disease progression.

Monocyte/MΦs exhibit functional and phenotypic heterogeneity, which is influenced largely by the surrounding cytokine environment (Gratchev *et al*, 2006; Park-Min *et al*, 2005; Porcheray *et al*, 2005). MΦs can exhibit immune polarization representing MΦs involved in promoting inflammation or 'proinflammatory MΦs' (type 1 MΦ or classically activated MΦ) and those involved in resolving inflammation or 'anti-inflammatory MΦs' (type 2 MΦ or alternatively activated MΦ) (Gratchev *et al*, 2006; Porcheray *et al*, 2005; Van Ginderachter *et al*, 2006). The CD163 positivity of the MΦs accumulating in the perivascular and parenchymal region in the CNS in HIVE and SIVE might suggest that these cells represent alternatively activated MΦs (Komohara *et al*, 2006). It is possible that these MΦs in the context of HIV and SIV infection exhibit a unique gene expression program. It is tempting, however, to speculate that alterations in monocyte/MΦ homeostasis (as illustrated by our immunohistochemical and flow cytometric studies) contribute not only to the

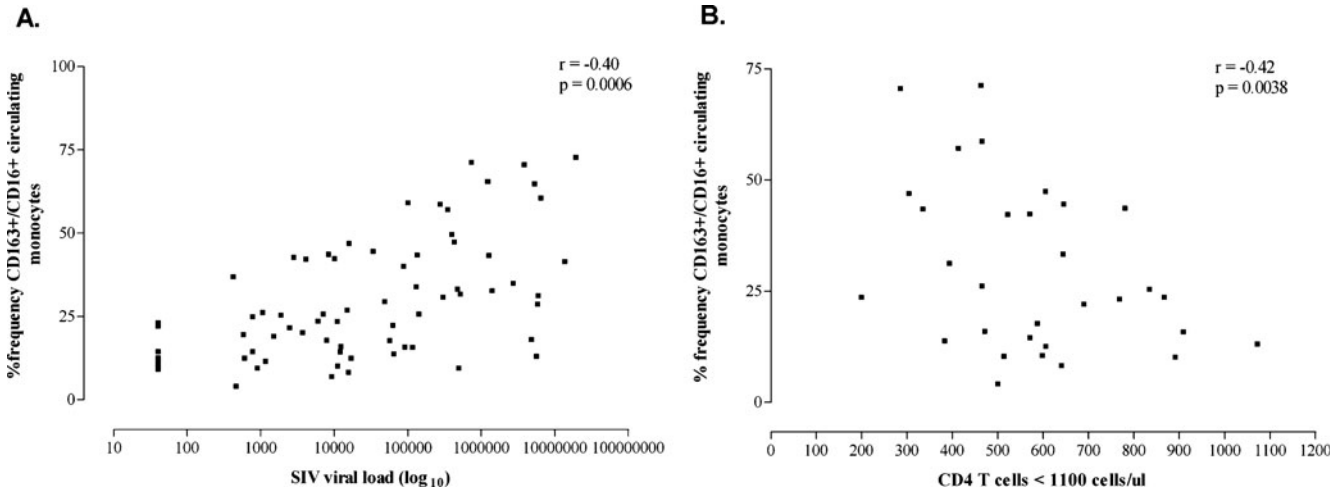
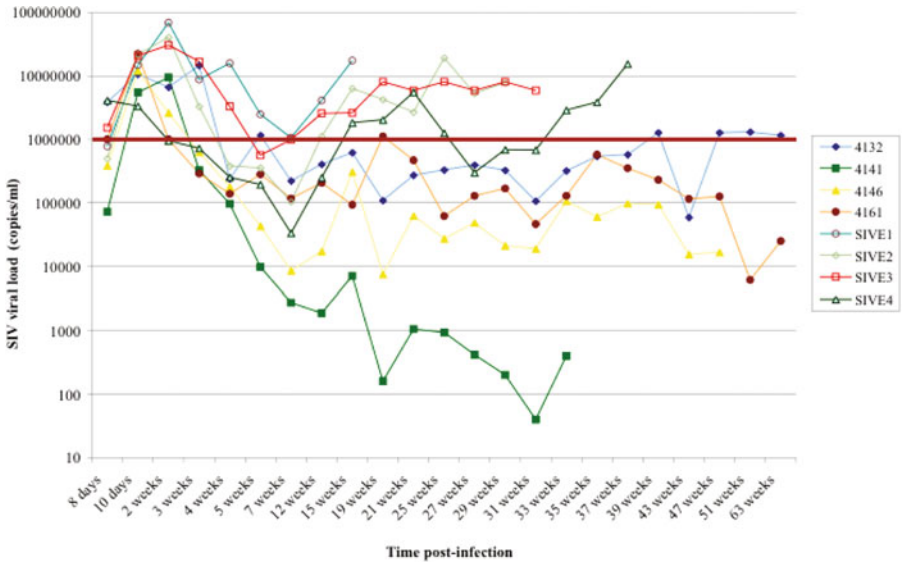


Figure 3 The frequency of $CD14^+/CD163^+/CD16^+$ monocytes is increased in SIV-infected animals, with detectable viral loads and correlates with viral burden and $CD4^+$ T-cell decline. Flow-cytometric studies showed that the percent frequency of $CD163^+/CD16^+$ monocytes ($CD14^+$) from SIV+ animals with detectable virus correlates with viral load (**A**). An inverse correlation was observed between the percent frequency $CD14^+/CD163^+/CD16^+$ monocytes and absolute number of $CD4^+$ T cells in animals with counts less than 1100 cells/μl (**B**). Together, these data suggest that expansion of this monocyte subset may contribute to and/or result from increased virus production and AIDS progression ($CD4^+$ T-cell loss). One-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons post test and correlation tests were performed on flow cytometry data using Graph Pad Prism version 3.00 software, San Diego, CA.



Graph 1 Development of CNS disease is associated with high viral loads in SIV-infected rhesus macaques. All data points show viral load (copies/ml) at designated times post infection. Animals with closed data points did not demonstrate encephalopathy at necropsy. Animals with open data points demonstrated pathology consistent with SIVE at necropsy. SIVE was seen predominantly among animals whose viral loads generally remained at 1×10^6 copies/ml (purple line on graph) or greater during the course of disease.

pathogenesis of HIV in CNS, but also to T-cell immune dysfunction in HIV infection leading to AIDS. It is interesting to note that in previous studies using a recombinant SIV/HIV virus designated SHIV ku-2, increased MΦ tropism (despite CXCR4 utilization) was associated with increased virulence, CD4 depletion, and CNS and renal diseases (Liu *et al*, 1999). Additional studies will be required to characterize the phenotypic and functional characteristics of monocyte/MΦs in the context of HIV and SIV infection. Such studies should open additional avenues for therapeutic intervention.

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