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Monocyte/macrophages and their role in HIV neuropathogenesis

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Summary

Neurologic sequelae of human immunodeficiency virus (HIV) infection have been and remain a significant problem. Monocytes and macrophages in humans and monkeys are susceptible to infection by HIV and simian immunodeficiency virus (SIV) and are considered to be a main mechanism by which the central nervous system (CNS) is infected. Within the infected CNS, perivascular macrophages and, in some cases, parenchymal microglia are infected as are multi-nucleated giant cells (MNGC) when present. While neurons are not themselves directly infected, neuronal damage occurs within the infected CNS. Despite the success of anti-retroviral therapy (ART) in limiting virus in plasma to non-detectable levels, neurological deficits persist. This review discusses the continued neurological dysfunctions that persist in the era of ART, focusing on the roles of monocyte and macrophage as targets of continued viral infection and as agents of pathogenesis in what appears to be emergent macrophage-mediated disease resulting from long-term HIV infection of the host. Data discussed include the biology of monocyte/macrophage activation with HIV and SIV infection, traffic of cells into and out of the CNS with infection, macrophage-associated biomarkers of CNS and cardiac disease, the role of antiretroviral therapy on these cells and CNS disease, as well as the need for effective adjunctive therapies targeting monocytes and macrophages.

Keywords

monocytes/macrophages; immunodeficiency diseases; AIDS

Introduction

Human immunodeficiency virus (HIV) infection of the central nervous system (CNS) has been and continues to be a significant problem. Monocytes and macrophages in humans and monkeys are susceptible to infection by HIV and simian immunodeficiency virus (SIV) and are considered to be a main mechanism by which the CNS is infected (1). Within the infected CNS, perivascular macrophages and, in some cases, parenchymal microglia are infected as are multi-nucleated giant cells (MNGC) when present (2). While neurons are not themselves directly infected, neuronal damage occurs within the infected CNS. This damage leads to neuronal damage and loss, glial cell and macrophage activation, and neurological impairment. Early studies using anti-retroviral therapy (ART), with successful reduction of plasma virus, demonstrated a rapid and profound reversal of HIV-associated dementia (HAD) (3). Unfortunately, over the years it has become clear that while successful ART can limit virus in plasma to non-detectable levels, neurological deficits persist (4–7). Overall, the

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incidence of HAD has decreased, but the prevalence of neurological deficits have increased. This increase in neurological defects is due in part to people living longer with virus, but the patients with neurological defects have less severe neurological dysfunction than prior to ART (4, 5, 8–10). In this setting, it is becoming clear that patients on effective ART who have non-detectable plasma virus have persistent immune activation that includes monocytes and macrophages, accelerated immune aging and senescence, and other co-morbidities including an increased incidence of coronary atherosclerosis, kidney disease, osteoporosis, and liver disease. These phenomena may be due in part to the effects of anti-retroviral agents used, but they also are likely driven by chronic infection and activation of innate immunity. This review discusses the continued neurological dysfunctions that persist in the era of ART, focusing on the roles of monocyte and macrophage as targets of continued viral infection and on agents of pathogenesis in what appears to be emergent macrophage-mediated disease resulting from long-term HIV infection of the host. Data discussed include the biology of monocyte/macrophage activation with HIV and SIV infection, traffic of cells into and out of the CNS with infection, macrophage-associated biomarkers of CNS and cardiac disease, the role of antiretroviral therapy on these cells and CNS disease, as well as the need for effective adjunctive therapies targeting monocytes and macrophages.

The status of neurological impairment with HIV infection and ART – incomplete viral suppression with immune activation drives disease

Antiretroviral therapy has had a dramatic beneficial impact on the incidence and prevalence of severe forms of HIV-associated neurocognitive impairment (3). Because of this, HIV-associated dementia (HAD) has become rare. The estimated prevalence of HAD in the pre-ART era was between 10 to 15% of HIV infected individuals, and in the current era this has decreased by over 50% (4, 5, 9, 10). While there is a decreased incidence of HAD, in the setting of chronic suppressive treatment, minor forms of neurocognitive impairment have emerged (11). Current research nosology defines the broad spectrum termed HIV-associated neurological disorders (HAND) with graded classifications based on abnormal neuropsychological (NP) testing (a battery of 7 tests) and the patient's functional limitation related to cognitive impairment. The three classifications of HAND are asymptomatic neurocognitive impairment (ANI), mild neurocognitive impairment (MND), and HIV-associated dementia (HAD) (11). ANI is defined as acquired impairment in at least two cognitive domains without perceived impact on daily function. MND requires impairment in two domains with perceived interference of daily functioning. HAD requires at least 2 impaired domains with severe impairment, typically in multiple domains, and marked impact on daily function (11).

Thus, HIV-associated CNS injury and neurocognitive impairment continues to be clinically significant in the current era of ART (5). Overall, studies demonstrate NP benefits of instituting ART in patients with HAND (3, 6, 7, 12), but in many cases HAND persists (11, 13–15). The persistence of HAND is likely mediated by continual immune activation that includes monocytes and macrophages. Another likely cause for continued neurologic deficits is that many ART-treated patients have some level of viral replication above non-detectable levels (< 50 copies/mL) (4, 16). In addition to continued detectable HIV in plasma, CSF virus can also persist (4, 16–19), which might also play a role in CNS disease. It is very possible that extended survival with incomplete viral suppression plays a role in the persistence of HAND.

Early neuronal injury with HIV and SIV infection, with and without ART

The mechanisms of HAND persistence in the ART era are not well understood. It is likely that events occurring early in the CNS set the stage for a future neurological disease that emerges during chronic infection with or without effective ART. Neuroimaging studies in monkeys and humans have revealed lower N-acetyl aspartate (NAA) levels, indicative of neuronal injury, within weeks of HIV and SIV infection (20–22). This observation is consistent with studies demonstrating early infection of the CNS days after infection (1, 23–26). Recently, it has been determined that the resting cerebral blood flow reductions that occur soon after HIV infection are reflecting early neuronal or vascular injury among HIV-positive individuals who do not yet manifest neuropsychological impairment (27). These imaging studies suggest that there exist early metabolite and blood flow changes, even though these changes do not result in significant clinical NP changes at those early time points.

Early initiation of ART: treatment within 1 year of infection effectively reduces innate immune activation

Early initiation of ART, before $CD4^+$ T cells decline, may have long-term cognitive benefits and help achieve a better neurological outcome. Our studies (28) using plasma soluble CD163 (sCD163) as a marker of monocyte macrophage activation have shown that for individuals with early HIV infection (≤1 year in duration), effective ART resulted in decreased sCD163 to levels that were comparable to normal HIV-seronegative individuals (29). However, in chronically infected HIV patients, sCD163 levels decreased in parallel with HIV RNA levels but did not return to HIV-seronegative levels, suggesting the presence of residual monocyte/macrophage activation even with plasma viral loads below the limit of detection (29). These data suggest that early initiation of ART, in addition to preserving $CD4^+$ T cells and decreasing viral load, would also dampen monocyte/macrophage activation. In addition to the studies described above, studies of patients on scheduled ART interruption during early HIV infection found that sCD163 and plasma virus levels spiked but rapidly returned to baseline with re-initiation of ART. These data suggest that not only early initiation but also continued treatment is necessary for effective inhibition of HIV, monocyte activation, and $CD4^+$ T-cell numbers (29).

Biomarkers/legacy markers

Legacy markers are defined as markers of events happening early in HIV infection that may lead to neurocognitive impairment in later stages of the disease and, in some cases, are considered to be predictors of disease (5, 30). Thus, patients with a history of early severe immunosuppression may have an increased risk of the development of HAND, even after effective ART and immune recovery. It is possible that advanced immunosuppression reflected by low $CD4^+$ T-cell nadir is a legacy event that may have occurred early and whose neurologic consequences may persist. The risk of neurocognitive impairment is lowest in patients whose $CD4^+$ T-cell counts were never allowed to fall to low levels before ART, and these patients were relatively protected from NP impairment (5, 30). These observations reiterate the importance of early initiation of ART in reducing the risk of neurocognitive impairment as well as the importance of identifying HIV-seropositive patients early and encouraging ART use to prevent later complications (30). Unfortunately, epidemiologic data from the Centers for Disease Control (CDC) indicate that it is often difficult to identify HIV infection in its early stages; at least 34% of patients in the US have a $CD4^+$ T-cell counts below 200 cells/μl at diagnosis (31). In addition to $CD4^+$ T-cell nadir, others have looked at viral set point as an early predictor of HAND (32). Such legacy

markers are likely markers of differential immune responses that reflect levels of immune activation and predict future HAND among HIV-infected individuals.

There is no biomarker of HAND that is used currently in the clinical setting that correlates to HAND in the era of ART. Early work in monkeys and humans indicated that CSF markers including HIV RNA (33) and immune activation factors [β2-microglobulin (34, 35), neopterin(36, 37), sCD14 (38, 39) and CCL2/MCP-1 (40–43)] were diagnostic of HAD (33, 44). Unfortunately, these markers are not as effective in patients on ART (33, 45). Our work has shown that NP impairment in ART treated and virologically suppressed HIV-infected individuals is associated with a sustained elevation of the monocyte/macrophage activation marker sCD163 in plasma (46, 47). Interestingly, CD163 is made solely by cells of the myeloid lineage and shed by them with immune activation. Our data (described above) demonstrate that plasma sCD163 is an early marker that predicts HIV/SIV pathogenesis (29). We reported elevated levels of sCD163 in patients with impaired global deficit scores (GDS) and in patients with HAND with persistent monocyte/macrophage activation in HIV-related NP impairment despite virologic suppression(46, 47). These data underscore the utility of sCD163 as a plasma marker of neurocognitive impairment that correlates to HAND in individuals on ART. We did not find correlations of sCD163 with NP impairment, unlike other biomarkers described previously(46, 47). Whether sCD163 levels in plasma are predictive of NP impairment will require studies with larger numbers of patients.

The observations above underscore the importance of monocyte activation and NP impairment with HIV infection, even with effective ART. We, along with others, have implicated monocyte activation and traffic not only as a source of inflammatory cells in the CNS and a key mechanism for neuroinvasion but also as a requirement for CNS pathogenesis (48–53). Such a perspective includes the notion that monocyte traffic to the CNS, which occurs normally in non-diseased brains, occurs with acute and chronic infection, and the accumulation of these cells as transient or resident macrophages contributes to immune dysregulation and disease (48–53) (discussed below). To this end, we found that differences with sCD163 in monkeys infected with SIV, as early as 8 days post infection and consistently by 21 days post infection, that predicted how rapidly these monkeys would develop AIDS and how severe CNS pathogenesis would be (53). sCD163 in these monkeys directly correlated with the magnitude of monocyte turnover in the bone marrow. Taken together, these data point to a link between early innate and adaptive immune response and future CNS pathology.

Mechanisms of HIV CNS disease pathogenesis: monocyte traffic and accumulation as transient or resident brain macrophages

Exact mechanisms for neurological impairment caused by HIV and SIV infection remain elusive. Many factors have been hypothesized including the virus itself, which does not infect neurons but does interact with neuronal cells, immune cells and glial cells by virtue of their viral proteins (reviewed in 54). Additionally, factors released from activated glia, including parenchymal microglia (the resident macrophage in the CNS), and inflammatory macrophages that are recruited also likely play a role (reviewed in 55). Early literature suggested the presence of virus alone was the best correlate of neuronal injury and dysfunction(56), but more recent evidence points to the accumulation of macrophages as the histopathologic correlate of HIV-associated neuronal dysfunction (57, 58). This seems also to be true in the era of ART, although less extensive histopathologic data exists. Consistent across all of the possible mechanisms is a prominent role for activated monocytes and macrophages.

Expansion and turnover of monocyte blood populations

The earliest evidence of the link between activated monocytes and HAD came from Drs. Pulliam and McGrath (59), who showed a correlation between the presence and number of $CD14^+CD16^+$ monocytes and HAD in HIV-infected men. Others have observed a similar expansion of $CD14^+CD16^+$ monocytes in HIV-infected humans (49, 60, 61) and SIV-infected monkeys (48, 62). The population of $CD14^+CD16^+$ monocytes are of interest with regard to HIV infection, because they are more susceptible to HIV infection (49, 62–64) and have an immune phenotype and possible functions that are more similar to mature macrophages than monocytes found in blood (1, 49). Whether this maturation accounts for their enhanced ability to replicate HIV is unknown. In HIV and SIV infections, there are also increased numbers of activated monocytes (1, 49, 59, 62, 65) and turnover of monocyte/macrophages in tissues (53, 66). Kuroda and colleagues first demonstrated in SIV-infected monkeys, using bromodeoxyuridine (BrdU), an analogue of thymidine that is incorporated in DNA during replication, that an increased turnover of monocytes from the bone marrow is a better marker of progression to AIDS than $CD4^+$ T-cell count and plasma viral load (66). Extending those observations, Burdo *et al.* (53), also using BrdU incorporation, found that the magnitude of $BrdU^+$ monocytes in blood of SIV-infected monkeys predicted how rapidly they would progress to AIDS. The more $BrdU^+$ monocytes that were found in the blood of the animals (indicative of increased turnover), the more severe the brain histopathology (macrophage accumulation and productive viral infection) (53). Rappaport and colleagues (60) made similar observations, finding an expansion of $CD16^+CD163^+$ monocytes in blood that may replace or take up residence in the CNS of HIV-infected individuals. It is likely that this same increased monocyte turnover and accumulation of macrophages in the CNS is occurring in other organs, including the heart, within HIV-infected individuals even with durable ART.

Several recent reports have described an activated monocyte phenotype in HIV-infected patients with cardiovascular disease that is positive for CD14, CD16, and tissue factor (TF) that is associated with activation of coagulation factors and that correlates with reduced intima of cardiac vessels, increased vulnerable non-calcified plaque, and macrophage accumulation in the ascending aorta (67–74). In addition, there is evidence of elevated C-reactive protein (CRP), d-dimer, platelets, and microparticles underscoring chronic and sustained immune activation and activation of coagulation factors that can contribute to cardiac and CNS inflammation and dysfunction (75–79). It is important to note that accumulation of $CD68^+$ macrophages that are also $CD163^+$ has been demonstrated in cardiac tissues of SIV-infected monkeys (80, 81) as well as in the CNS (53, 60, 82–84). It is also noteworthy that elevated sCD163 cleaved from activated monocytes in HIV-infected humans with coronary atherosclerosis (69) and neuroAIDS (46, 47, 53) correlates with the number of inflammatory macrophages in hearts (85), HIV activity prior to and with effective ART (29), and neurocognitive deficits (46, 47). Thus, activated monocytes, and sCD163 as a marker of them, are minimally a biomarker of cardiac and CNS disease in HIV-infected patients, and macrophages in the heart and CNS are likely effector cells with regard to immune and physiologic responses to HIV infection. This observation suggests that in addition to developing therapies that target HIV, it would be important to also target monocyte/macrophage activation and traffic to affected organs.

CNS macrophage phenotypes

Our group and others (1, 49, 53, 60, 62, 83, 84, 86, 87) have demonstrated that populations of CNS macrophages can be distinguished by a combination of their anatomic location in the CNS and immunophenotypic markers. With regard to HIV and SIV infection, we have studied the resident parenchymal microglia, perivascular macrophages, and inflammatory

macrophages that are only present in the CNS with inflammation (50, 88, 89). Perivascular macrophages are $CD14^+CD16^+CD163^+$ and have high levels of CD45 (1, 83, 84, 86, 90, 91). Parenchymal microglia have low to non-detectable CD14 and CD16. These two cells types are the major constituents of HIV and SIV encephalitic lesions (1, 52, 83, 86, 92), where productive viral replication is often found. Perivascular macrophages are also found in small lesions along CNS vessels where they are infected (1, 83, 93). Another monocyte/macrophage cell type is present with inflammation. They are distinguished from perivascular macrophages and parenchymal microglia by being positive for MAC387 (84, 94). Comparing the numbers of $MAC387^+$ cells in the CNS of (i) chronically SIV-infected monkeys with and without SIVE, (ii) rapid progressing animals with SIVE, and (iii) $CD8^+$ lymphocyte-depleted animals with SIVE, we find $MAC387^+$ cells are present in early developing lesions that are still immune active. This finding is similar to what has been found in multiple sclerosis (84). Interestingly, in our experiments using BrdU to examine cell trafficking to the CNS we find that, 24–48 h post BrdU pulse, the majority of the $BrdU^+$ monocyte/macrophages in the CNS are $MAC387^+$ cells. This finding is consistent with the notion that MAC387 is the earliest marker found on macrophages as they enter the CNS (53). In fact, when we compared the ratio of $MAC387^+$ monocyte/macrophages with the ratio of $CD68^+CD163^+$ macrophages, we found greater numbers of $MAC387^+$ cells than $CD68^+CD163^+$ cells in lesions that are early and active. Conversely, a greater number of $CD68^+CD163^+$ cells than $MAC387^+$ cells were present in chronic established lesions (84). We have identified a similar change in the ratio of $MAC387^+$ and $CD68^+CD163^+$ cells in the CNS tissues of HIV-infected humans (84). $CD68^+CD163^+CCR2^+$ perivascular macrophages have an M2-polarized alternatively activated macrophage phenotype, while the $MAC387^+$ cells are $CD163^-CCR2^-$, similar to classically activated M1 macrophages, which are thought to amplify immune and inflammatory responses and are associated with higher suppression of HIV-1 replication (95). It is tempting to suggest that the early lesions made up M1 macrophages represent an ongoing immune response to infection, while the later more chronic lesions made up of M2 macrophages may indicate attempts to downregulate immune responses (84, 96). In an extension of this notion, one could envision $CD163^+$ macrophages at the lesion edge functioning to contain the damaging immune responses within the lesion from the rest of the CNS.

Monocyte/macrophage turnover in the CNS

It is well established in rodents, nonhuman primates, and humans that monocytes traffic to the CNS occurs normally and in disease (97–101). Early classic studies demonstrated that parenchymal microglia are present embryologically from yolk sac macrophages and are distinct from other macrophage populations (102–104). The parenchymal microglia remain a relatively stable population with a low level of turnover within the CNS (105–107). These cells appear to remain tethered within a reticular network within the CNS and are able to scan their respective territories acting as sentinels protecting against damage, invading pathogens, and tumors (108). Macrophages of the choroid plexus and meninges, as well as perivascular macrophages, are bone marrow-derived and are repopulated throughout life (100, 105, 109, 110). These cells are more likely to serve as carriers for viruses and bacteria entering and perhaps exiting the CNS. Studies in rodents and humans have established that the perivascular macrophages are continuously repopulated from bone marrow (100, 105, 109). Using nonhuman primates, who were irradiated and reconstituted with retroviral transduced, autologous $CD34^+$ hematopoietic stem cells, we demonstrated turnover of perivascular macrophages that corresponded to the rate of engraftment of the stem cells in bone marrow and monocyte precursors in blood (111). Whether such cells could be engineered in a way that would make them not susceptible to HIV infection is not known, but it is an exciting possibility (112, 113).

Viral sequence in the CNS

The phenotype of CNS macrophages is relatively well established and critical with regard to which cells come to the brain and when and to which cells are important in terms of viral infection. Sequence analysis supports the notions that (i) virus found in the CNS is macrophage-tropic, meaning it replicates well in macrophages (114–116), (ii) virus in the CNS can be compartmentalized compared to other infected organs, but macrophage-tropic sequences in the CNS do match that of other organs, and (iii) there is evidence of early seeding of the meninges of the CNS, and then other brain tissues, suggesting successive waves or spread of virus, likely from infected monocytes that have become macrophages in the CNS (25, 58, 93, 116, 117). Importantly, evidence in the monkey model can support that notion that the CNS receives waves of viral seeding from the periphery that is not a one-time event but likely occurs throughout the course of infection (114, 116, 118). All of the above underscore the importance of myeloid cells as targets of infection outside and inside the CNS.

Drug treatment to stop virus and/or macrophage traffic: studies from SIV-infected macaques

Evidence from our laboratory and others using ART, minocycline, and antibodies directed against monocyte traffic to the CNS provides compelling evidence that continued monocyte activation in the periphery and traffic to the CNS, throughout infection drive the neuropathogenesis of HIV/SIV infection. Early studies by our group used a combination of 2 anti-retroviral agents, 9-R-2-phosphonomethoxypropyl adenine (PMPA) and racinavir (RCV), in an accelerated model of AIDS neuropathogenesis involving $CD8^+$ cell depletion where we demonstrated large decreases of NAA/Cr by MR spectroscopy consistent with neuronal injury at 28 days post infection (119). In CD8-depleted animals treated daily with PMPA and RCV, neither of which significantly penetrate the CNS, the decreased NAA/CR ratios reversed within 2 weeks on ART and returned to within 90% of pre-infection levels by 4 weeks post treatment (119). Within the CNS of these animals, there was little-to-no productive viral infection and few inflammatory macrophages in perivascular cuffs (119). The most significant marker that correlated with the reversal of neuronal injury and the paucity of productive infection and inflammatory cells in the CNS of treated animals was the lack of a second peak of expansion of activated monocytes in the blood of these animals (119). More recently, we investigated the use of the antibiotic minocycline, which has been shown to have anti-inflammatory properties, using the same $CD8^+$ cell depletion model and MR spectroscopy imaging studies (120, 121). Again, animals received minocycline daily beginning at day 28-post infection, a time point when we found significant loss of NAA/Cr and known active CNS inflammation. Minocycline treatment did not cause a restoration of NAA/Cr toward normal levels but did prevent subsequent declines in NAA/Cr, which we think is indicative of neuronal protection (120, 121). Moreover, minocycline treatment also resulted in decreased monocyte activation in blood, lack of traffic in an *ex vivo* transwell assay, reduced recruitment of macrophages to lymph nodes, and an absence of detectable inflammation and active viral replication in the CNS (120). Because neither of the studies used agents to prevent traffic of monocytes into the CNS, we performed a third study using an anti-VLA-4 antibody, and treated $CD8^+$ lymphocyte-depleted animals at the time of infection, once a week for 3 weeks, or at 28 days post infection and sacrificed animals one week after the final anti-VLA-4 treatment. This treatment resulted again in reversal of neuronal injury as measured by MR spectroscopy, immunohistochemistry, and neuropathological assessment in animals treated beginning 28 days post infection (122). Interestingly, in the animals that received treatment starting at the time of infection, not only was traffic of monocyte/macrophages and virus to the CNS blocked, but we also observed greatly diminished viral infection and traffic of monocytes to the gut (122). These data

therefore not only demonstrate that blocking monocyte and lymphocyte traffic to the CNS results in a reversal of CNS injury but that such treatment results in lack of seeding of the CNS with virus and less virus in the gut. This may be a critical short-term treatment to consider for individuals who engage in high-risk behavior. It could be used with ART to not only keep viral replication in check and spare the immune system (123) but also to potentially stop the seeding of important cellular reservoirs of virus in the CNS and gut. Additional data on the impact of changes in viral distribution and tropism on HIV/SIV pathogenesis come from recent work using the highly virulent molecular clone SIVmac239, in which two amino acids in the transmembrane protein were deleted (124). This virus, termed SIVmac239ΔGY, replicated to wildtype levels during acute infection, but only transiently infected mucosal sites such as the gut and caused little or no acute depletion of mucosal $CD4^+$ T cells and little if any infection of macrophages; CNS lesions were also absent (124). While the data noted above support the importance of monocyte/macrophages contributing to CNS infection and the neuropathogenesis of AIDS, similar targeted therapies using different outcome measures have not been successful. Namely, minocycline used in HIV-infected patients did not have a favorable impact on cognitive function (125, 126). Whether minocycline used in conjunction with ART might be beneficial is not yet known.

Synergistic effects of chronic immune activation, metabolic syndrome, acute phase proteins, cardiovascular disease, and CNS pathology during HIV infection

The above discussion underscores the importance of monocytes as major players and biomarkers of macrophage-mediated disease in HIV infection, including the pathogenesis of cardiac and CNS disease and NP deficits. Clearly, there are other comorbidities associated with HIV disease progression and HAND, including other infections and the likely emergence of degenerative diseases as HIV-infected populations age (e.g. Alzheimer's disease, Parkinson's disease, cardiovascular disease) (127). In this regard, it is important to note that HIV affects cholesterol metabolism by monocyte/macrophages, which has been described as contributing to fatty macrophages in the vasculature in HIV-infection (128). Additionally, HIV-infected patients have accelerated senescence of the immune system for reasons that are not clearly defined (129, 130). In addition to HIV-associated $CD8^+$ T-cell senescence, recent work has shown CD8-independent age-related changes in monocytes (129) and increases in markers of immune activation, sCD163 and CXCL10, in plasma (131). These changes suggestive of innate immune dysfunction and age-related immune senescence persist despite viral suppression (129). Several of these changes may be a result of generalized immune activation associated with microbial translocation (132), but other as yet unknown factors also probably contribute. A recent example of this is that rhesus macaques infected with SIVmac239ΔGY do not show any evidence of microbial translocation, and yet they develop immune activation and slow disease progression (124).

The lipopolysaccharide (LPS) and other microbial products from the gut drain into the liver, where the liver releases proteins associated with the acute phase response such as CRP, d-dimer, and IL-6. Additionally, platelet production and coagulation factors are increased, as well as activation of monocytes/macrophages and enhanced sCD163 shedding. In addition to chronic immune activation, which clearly affects monocyte/macrophages in HIV-infected patients, it is likely also due to secondary infection, residual low-level viral infection, and the effects of viral proteins on the immune system (133–135). There are reported correlations with body mass ratio, cardiovascular disease, and dementia with and without HIV (127, 131). All in all, it is clear that persistent macrophage activation is at least a marker of disease and CNS pathology, if not a contributor.

In this regard, it is interesting to consider our studies using $CD8^+$ cell-depleted SIV-infected monkeys. We found a linear correlation between the number of $BrdU^+$ monocytes in the blood 24 h after BrdU administration and the level of plasma sCD163 (53). The differences in the absolute number and percent of $BrdU^+$ monocytes in blood and the levels of sCD163 among monkeys that distinguished rapid versus slow progressor could be detected and used to differentiate between animals by 21 days post infection, despite that fact that it was weeks to months longer before the animals developed AIDS (53). Moreover, we have made similar observations in HIV-infected individuals with sCD163. Chronically infected patients had high levels of sCD163 that correlated with their $CD14^+CD16^+$ monocytes, which were reduced with effective ART, but not to the level found in uninfected patients (29). In contrast, sCD163 levels in the early infected patients were lower than that of the chronic patients, and with 3 months of effective ART, it decreased to the same level found in uninfected individuals (29). These data with the observations discussed earlier that sCD163 correlates with the percentage of non-calcified plaque in HIV-infected patients (69), the number of macrophages in the ascending aorta (71) and HAND (46, 47) demonstrate two points: one, it is best to treat with ART early after infection to essentially spare chronic, reversible monocyte/macrophage activation, and two, chronic immune activation of monocytes, as measured by sCD163, is a good marker of HIV activity and macrophage inflammation in HIV cardiac disease and HAND (29, 46, 69–71).

Mechanisms accounting for persistence of neurological disease with ART

It is clear that despite the advent of ART, which is capable of driving peripheral viral load to undetectable levels, neurological deficits persist. This is supported not only by the increasing incidence of HAND but also by histologic examination of tissues where inflammation is still evident, as are signs of immune activation. The reasons for persistent neurological disorders can be many, including the fact that ART is not absolutely effective in all patients and that residual chronic viral replication outside the CNS in various tissues, including the intestinal track, has been described (136, 137). Additionally, CNS and CSF viral replication is found and can persist, even if there is non-detectable or low level replication in plasma (4, 16–19). Letendre and others (138) have suggested considering the ability of antiretrovirals to penetrate the CNS and effectively decrease replication in the CNS when developing ART regimens by utilizing a formula called CNS-penetration-effectiveness (CPE). A caveat of this work in general is the relative inefficiency with which ART functions on macrophages (139–141). Also, it assumes that only CNS virus drives neuropathology. Others have suggested that the antiretroviral drugs themselves contribute to neuronal injury and several comorbidities, including cardiovascular disease (142, 143). Lastly, effects of comorbidities, such as drug abuse and co-infections with viruses such as hepatitis C virus (HCV), cytomegalovirus (CMV), and polyomavirus (JC virus, progressive multifocal leukoencepalopathy), as well as other coinfections, such as toxoplasmosis, also likely play a role.

In addition to the factors listed above, it is clear that ART has allowed people to live much longer with HIV infection. These aging HIV-infected populations will have an increasing incidence of degenerative neurological diseases, which will impact and be impacted by the neuropathologic effects of HIV infection. Common to HIV infection and degenerative neurologic diseases such as Alzheimer's and Parkinson's disease is glial cell activation and low-level chronic inflammation with activated monocytes and macrophages, which are likely to have synergistic negative consequences for neurologic function (144). Lastly, in addition to CNS diseases associated with aging, is the increasing role of cardiovascular disease, increased body mass index, and vascular dementia as they all likely relate to HIV neuropathogenesis. Similar to the CNS, macrophage accumulation in the heart and cardiac vasculature has been associated with tissue damage (128). In addition, emerging data point

to altered coagulation and lipid metabolism that contributes to cardiovascular disease, which would also affect CNS vasculature and complicate HAND. All of these observations underscore the importance of adjunctive therapies to target immune activation in general and activated monocyte/macrophages in particular with regard to neurologic and cardiovascular disease.

Future therapeutic approaches to HIV and macrophage-mediated diseases

It is clear that future research should focus on modulating activation of monocyte/macrophages, which seems to be central to the longer term degenerative aspects of HIV infection in addition to the control of viral replication by ART. Few pharmacologic agents that modulate monocyte/macrophage activation are currently available. Minocycline, an antibiotic with anti-inflammatory properties, has been unsuccessful in clinical trials of HIV-infected individuals in treating HIV-associated cognitive disorders (125, 126). It is not clear why minocycline has not been used with patients in combination with ART. Minocycline has potential as an adjunctive therapy for HIV-1-associated cognitive disorders. Statins are clearly one class of drugs that have reported immune suppressive effects that might be beneficial in HIV-infected patients. Similarly, dimethyl fumarate, which has been shown to be an immune modulator and inducer of antioxidant response that suppresses HIV replication and macrophage-mediated neurotoxicity (145), might also be of benefit. Work in our laboratory using a newly developed oral form of MGBG, termed PA300, has been effective in SIV-infected monkeys and resulted in decreased monocyte/macrophage accumulation and prevented SIV encephalitis in CD8 animals treated at day 28 post infection for 4 weeks (146). The same drug, in a dose dependent manner, decreased inflammation in the hearts of infected monkeys and decreased damage to the cardiac tissues.

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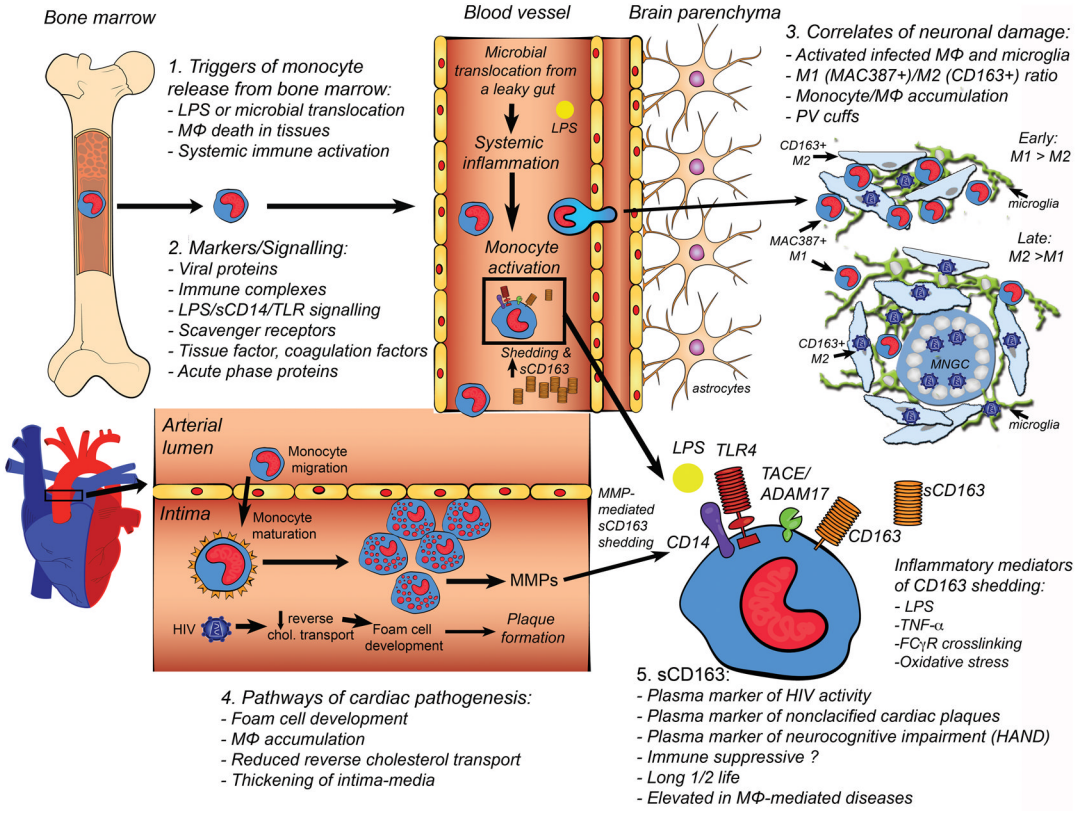

Fig. 1. Pathways and mechanisms of expanded monocyte/macrophage activation, turnover and accumulation in parenchymal tissues with HIV infection

1) Monocyte egress from bone marrow in response to elevated LPS, macrophage death in tissues (lymph node and parenchymal), and systemic immune activation. 2) Markers and signaling molecules involved in augmented monocyte activation and turnover, including viral proteins, acute phase proteins, immune complexes, sCD163 and signaling through LPS/CD14/TLR4 and macrophage scavenger receptor (SR-A), and expression of tissue factor (TF) and CD163. 3) Correlates of neuronal damage in the central nervous system include: activated and infected macrophages and parenchymal microglia, M1 (MAC387)/ M2 (CD163) ratio, monocyte/macrophage (MΦ) accumulation, and perivascular (PV) cuffs. MNGC= multi-nucleated giant cell. 4) Pathways of cardiac pathogenesis including development of foam cells, macrophage accumulation in the intima, reduced reversed cholesterol transport, thickening of the intima-media. 5) sCD163 is a marker of HIV activity, plasma marker of non-calcified (vulnerable) cardiac plaques, and neurocognitive impairment. sCD163 has immune-suppressive functions *in vitro*, is elevated in macrophage mediated diseases, has a long half-life, and is therefore a stable biomarker.