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MINIREVIEW

Macrophages in Progressive Human Immunodeficiency Virus/Simian Immunodeficiency Virus Infections

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The cells that are targeted by primate lentiviruses (HIV and simian immunodeficiency virus [SIV]) are of intense interest given the renewed effort to identify potential cures for HIV. These viruses have been reported to infect multiple cell lineages of hematopoietic origin, including all phenotypic and functional CD4 T cell subsets. The two most commonly reported cell types that become infected *in vivo* are memory CD4 T cells and tissue-resident macrophages. Though viral infection of CD4 T cells is routinely detected in both HIV-infected humans and SIV-infected Asian macaques, significant viral infection of macrophages is only routinely observed in animal models wherein CD4 T cells are almost entirely depleted. Here we review the roles of macrophages in lentiviral disease progression, the evidence that macrophages support viral replication *in vivo*, the animal models where macrophage-mediated replication of SIV is thought to occur, how the virus can interact with macrophages *in vivo*, pathologies thought to be attributed to viral replication within macrophages, how viral replication in macrophages might contribute to the asymptomatic phase of HIV/SIV infection, and whether macrophages represent a long-lived reservoir for the virus.

Macrophages are a diverse and functionally important component of the immune system. Evolutionarily conserved in almost all species of the phylum Chordata, macrophages are one of the "oldest" leukocyte lineages and have the highest degree of plasticity across leukocyte subsets (1). Macrophages differentiate from the yolk sac, fetal liver, and peripheral blood monocytes that developed from bone marrow-derived hematopoietic stem cells. With their phenotypic and functional plasticity and presence in disparate tissues, macrophages play supportive roles in multiple aspects of physiology. Their function often depends upon their anatomical location, their individual ontogeny, and extracellular cues. For example, macrophages derived from the yolk sac or fetal liver reside in organs such as the brain (as microglia cells), pancreas, spleen, liver (as Kuppfer cells), and kidney. For many years, yolk sac- and fetal liver-derived macrophages were thought to be very long-lived, perhaps for the life span of the host. Indeed, after differentiating during fetal development, these macrophages can populate tissues for the duration of the host's life. However, recent data suggest that these cells can also divide *in vivo* to maintain homeostasis (1–3) and are able to rapidly repopulate after chemotherapeutic depletion (in the case of brain-resident microglia cells [4]). Our understanding of macrophage longevity and factors important for their homeostatic proliferation *in vivo* is incomplete and more data are required.

FUNCTIONS OF MACROPHAGES IN HEALTH

Though macrophage longevity is not fully understood, many studies have demonstrated that tissue-resident macrophages have critically important functions in tissue immunity and repair, antigen presentation, and tissue homeostasis. Indeed, the importance of their functionality can be tested in mice *in vivo* by therapeutic administration of toxins that kill macrophages after toxin phagocytosis (5, 6). Of the many roles macrophages play in health and disease, the two most prominent functions are the production of effector cytokines and chemokines and the phagocytosis of pathogens, immune complexes, and dead or dying cells. The type of effector cytokines and chemokines produced after antigen recognition depends on the context in which the antigen is recognized. The molecules produced by macrophages can direct epithelial cell homeostasis, tissue remodeling, and/or recruitment of other leukocytes.

Though macrophages are a member of the innate arm of the immune system and lack genetically rearranged antigen receptors, they can modulate their function in response to environmental and antigenic cues. For example, macrophages express Toll-like receptors (TLRs) that allow quick response to individual microbial antigens after exposure. Moreover, cytokine receptors allow macrophages to modify their subsequent functionality. In particular, differential recognition of gamma interferon (IFN-γ), interleukin-4 (IL-4), IL-13, and individual TLR ligands is thought to allow macrophages to tune their functionality toward an M1 or M2 phenotype. M1 and M2 functional profiles have some similarity to Th1 and Th2 functions observed in memory CD4 T cells. M1 macrophages produce effector cytokines such as tumor necrosis factor (TNF) and IL-12 and thus help in differentiation of CD4 T cells toward a Th1 phenotype, whereas M2 macrophages produce effector cytokines, including IL-10, and are important for wound healing. Macrophage functionality is significantly more complex than this oversimplification and is an area of active investigation (7, 8).

FUNCTIONS OF MACROPHAGES IN HIV/SIV INFECTIONS

HIV infection of humans and simian immunodeficiency virus (SIV) infection of Asian macaque monkeys are associated with robust virus replication and progressive loss of CD4 T cells that

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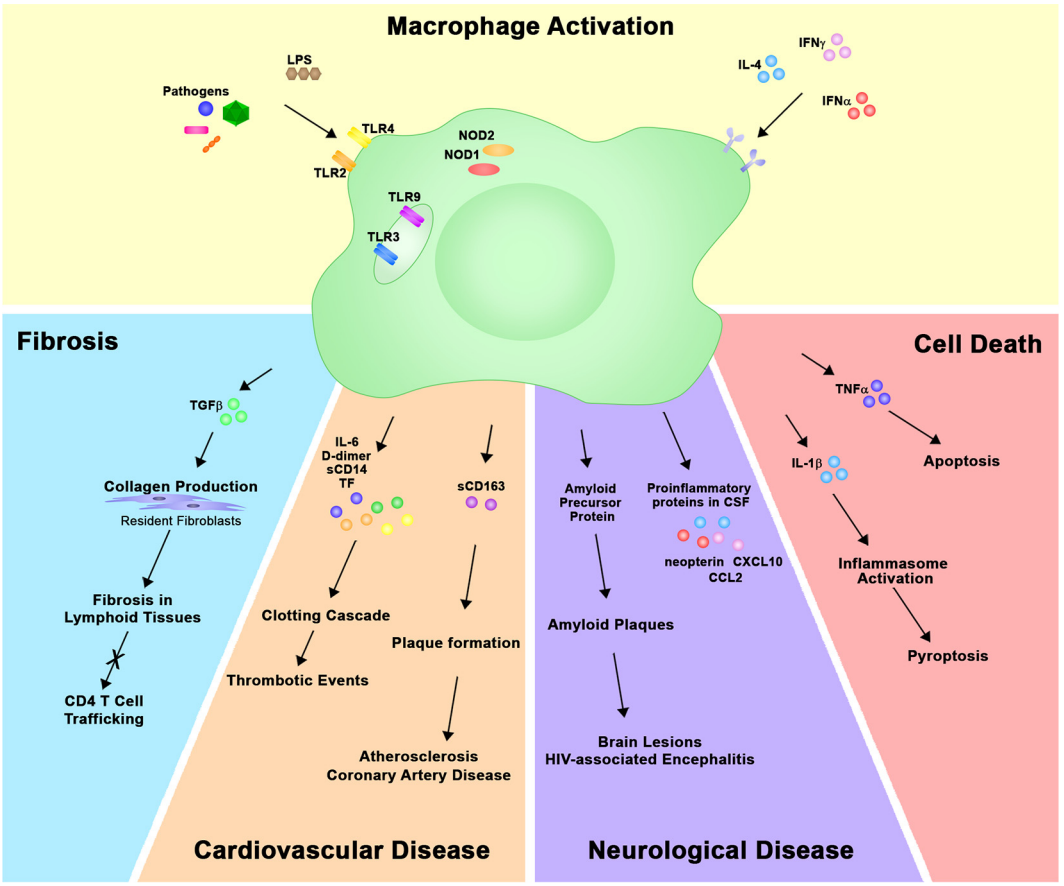

FIG 1 Macrophage activation and subsequent contributions to systemic inflammation and disease progression. Macrophages can be activated by pathogens, microbial products, or proinflammatory cytokines. Activated macrophages then contribute to inflammation-associated pathologies, including fibrosis, cardiovascular disease, neurological disease, and bystander cell death in surrounding tissues.

ultimately render infected individuals susceptible to opportunistic infections and/or neoplasias. While the virus directly infects CD4 T cells, leading to their death, HIV/SIV pathogenesis is complex, with multiple contributing factors. In addition to viral infection of CD4 T cells and their subsequent death, a hallmark of progressive immunodeficiency lentiviral infections is systemic inflammation. Importantly, the degree to which the immune system is stimulated is the best predictor of the rate of disease progression (9, 10). While the systemic inflammation observed in HIV/SIV infection includes every component of the immune system, activation of CD4 T cells in particular directly benefits the virus by increasing the pool of preferred target cells. The causes of inflammation observed in progressive HIV/SIV infections are many and are the center of much research effort (11). Broadly speaking, inflammation is thought to be initiated by factors including translocating microbial products from a damaged gastrointestinal (GI) tract, recognition of viral nucleic acids via Toll-like receptors, and responses to proinflammatory cytokines such as TNF-α and IFN-α.

Inflammation is an important factor in HIV/SIV disease progression in both untreated and antiretroviral (ARV)-treated individuals. Even in HIV-infected individuals receiving ARV treatment, residual inflammation persists. Several studies have shown that, especially if ARVs are initiated in the chronic phase of infection, this residual inflammation is associated with increased mortality among ARV-treated, HIV-infected individuals (12–14). Importantly, this increased mortality is not associated with opportunistic infections but instead is attributed primarily to cardiovascular disease and malignancies.

Tissue macrophage functions are thought to contribute to HIV/SIV disease pathogenesis via a variety of mechanisms. Macrophages are thought to be targeted by the virus *in vivo*, to respond to microbial products that translocate from the lumen of the GI tract, to phagocytose infected T cells, antibody complexes, and microbial products, and to remodel tissue (Fig. 1). While CD4 T cells, in particular memory CD4 T cells, are thought to be the predominant target for the virus *in vivo*, several studies have suggested that macrophages can also serve as targets for HIV and SIV (discussed in more detail below). During the chronic phase of HIV/SIV infection, and after administration of ARVs, myeloid cells clearly contribute to the observed systemic inflammation (Fig. 1). Though the antigens or factors that directly stimulate myeloid cells *in vivo* are multifactorial and incompletely understood, they likely include bacterial products, dead and dying cells, virus particle-containing immune complexes, and proinflammatory cytokines (15).

Irrespective of the antigens/products that directly stimulate myeloid cells *in vivo*, myeloid cell responses directly contribute to disease pathogenesis. The effector molecules produced by myeloid cells after stimulation include TNF-α, IL-1β, soluble CD14

(sCD14), D-dimer, IL-6, transforming growth factor β (TGF-β), tissue factor (TF), sCD163, and IL-13 (16–18). These molecules each play particular roles in pathogenic occurrences observed in HIV-infected individuals. TNF-α and IL-1β can have direct effects on cells within multiple tissues. TNF-α signaling through many receptors can lead to activation of proteases that activate intracellular caspase proteins, leading to apoptotic death (19). IL-1β can lead to activation of the inflammasome with subsequent induction of pyroptosis-mediated cell death (20). Exuberant production of these two effector cytokines can thus lead to bystander cell death that can contribute to HIV/SIV disease pathogenesis. Elevated levels of sCD14, TF, D-dimer, and IL-6 are observed in both ARV-treated and treatment-naive HIV/SIV-infected individuals (12, 14, 18). Importantly, in ARV-treated HIV-infected individuals, plasma levels of these markers are associated with increased mortality (11, 13, 21). As mentioned, a predominant cause of increased mortality is cardiovascular disease (CVD). IL-6, sCD14, TF, and D-dimer are thought to act directly on the platelet-clotting cascade, increasing thrombotic events and contributing to CVD (14, 18, 22, 23). Additionally, macrophage activation can lead to fibrosis. Stimulated macrophages can produce TGF-β, which is thought to lead to reorganization of extracellular spaces (24, 25). TGF-β stimulates production of collagen by resident fibroblasts. This collagen production is part of the wound healing process, but chronic collagen deposition leads to fibrosis (26). Fibrosis within lymphoid tissue of HIV/SIV-infected individuals is thought to limit the ability of the tissue to support healthy immunological processes (24, 25, 27).

Taken together, it is clear that the function of tissue-resident macrophages is critically important for health. However, their chronic activation can lead to several detrimental consequences that contribute to HIV/SIV disease progression, and novel therapeutic interventions might aim to blunt these responses.

CLINICAL DISORDERS ASSOCIATED WITH VIRAL REPLICATION IN MACROPHAGES

Prior to the development of ARV therapy, up to 60% of HIV-infected individuals were diagnosed with a disorder referred to as HIV-1-associated dementia (HAD) (28–31). The term HAD is somewhat ambiguous and includes any symptom involving cognitive impairments, which can range from psychiatric disorders to motor impairment. Decreased learning, information processing, and concentration or attention are also associated with HAD (31, 32). More severe disease can include HIV-associated encephalitis (33). While there are conflicting reports on potential effects of HIV-1 clade type on HAD incidence, HIV clade type may influence the likelihood that persons living with HIV will develop neurocognitive disorders and/or the severity of HAD onset and progression (34). For example, HIV-1 clade D virus is associated with a higher frequency of HAD than HIV-1 clade C (34). Confounding factors, including access to ARV therapy, neuropsychological diagnostic methodology, and coinfections, limit comparisons across these studies. Recent data suggest neurodevelopmental delay and/or acute neurological symptoms in young children infected with clade C HIV-1 (35). There is evidence that HAD is attributable to viral infection of brain-resident macrophages (microglia cells and perivascular macrophages), especially when HAD has advanced to HIV-associated encephalitis (33). In *in vitro* studies, these cells can be infected with certain HIV subtypes (36, 37). Moreover, viral RNA levels in cerebral spinal fluid (CSF) are elevated in HIV-infected individuals with HAD (38). However, viral RNA is only detected in ~20% of CSF samples of ARV-naive, HIV-infected individuals with peripheral blood CD4 T cell counts higher than 500 (39). Multinucleated giant cells, which are thought to develop after HIV envelope protein-mediated fusion of infected cells, are often found within the lesions observed in HIV-associated encephalitis (40, 41). However, it is important to note that giant, multinucleated cells are observed in many diseases associated with inflammation (independent of HIV/SIV infections), and this is thought to be attributable to fusion of adjacent macrophages in the presence of proinflammatory cytokines such as IL-4 and IL-13 (42). Thus, the degree to which the observed multinucleated cells in HIV/SIV infections can be attributed to envelope protein-mediated fusion versus inflammation-mediated fusion warrants further investigation.

While assessing direct viral infection of brain-resident leukocytes has remained somewhat elusive (discussed below), much effort has focused on understanding the quasispecies of contemporaneous viruses that exist in plasma and CSF. These studies have consistently demonstrated compartmentalization of HIV sequences within the CSF compared to virus in plasma (35, 38, 43). Similarly, viruses isolated from the brains of SIV-infected macaques with SIV encephalitis are genetically different from viruses isolated from other anatomical sites (44), and host genetic factors are thought to contribute to the ability of SIV to become neurotropic (45). Moreover, viruses isolated from the CSF infect macrophages efficiently *in vitro*, and their envelopes tend to have a higher affinity for CD4 (46–48). Further, some studies have analyzed brain tissue taken from HIV-infected individuals postmortem. Using molecular approaches to detect viral RNA and DNA, these studies suggested that individuals who were suffering from some neurocognitive deficiencies had higher levels of viral nucleic acids in the brain (49, 50). Additional studies using immunohistochemical analysis with cell-specific markers in combination with HIV-specific *in situ* hybridization or antibodies against HIV proteins have suggested that the virus exists within brain microglia and perivascular macrophages (40, 51). Though replication-competent virus has been isolated from brain tissue sections, more definitive studies with isolation of viable perivascular macrophages or microglia cells and subsequent recovery of replication-competent virus have not been performed. Hence, direct and conclusive evidence that virus within the CSF originates from brain-resident myeloid cells is lacking.

With the advent of highly active antiretroviral therapy (HAART), the severity of HAD has significantly decreased (34, 52, 53). However, subtler neurological disorders can arise even in individuals who are aviremic with HAART (54). These milder neurological disorders have been collectively designated HIV-associated neurocognitive disorder (HAND). Though the frequency of HAD has significantly decreased with HAART, approximately half of all HIV-infected individuals develop some form of HAND (34, 55, 56). One plausible explanation for HAND is decreased, but incompletely suppressed, viral replication within the central nervous system (CNS) (54, 57). Indeed, the blood-brain barrier is thought to decrease the penetrance of antiretroviral medication into the CNS (55, 58). Hence, suboptimal dissemination of ARVs into the CNS might allow virus to replicate at low levels within brain-resident cells, resulting in HAND. Some studies have shown that viral nucleic acids can be recovered from the CNS of individuals who are treated with antiretrovirals and who have contempo-

raneously undetected virus in plasma (59). While residual viral replication in brain-resident cells could contribute to neurological diseases observed in ARV-treated individuals, several studies have also implicated local inflammation (33, 52, 60, 61). Myeloid cells produce multiple proinflammatory proteins that have been directly implicated in HAND *in vivo*. Levels of neopterin, CXCL10, and CCL2 are elevated in the CSF of individuals with HAND, suggesting that infiltration and activation of local myeloid cells may contribute to the disease (62). Moreover, inflammation is thought to promote production of amyloid precursor protein, potentially leading to formation of amyloid plaques—which are observed in individuals with neurological symptoms (63)—and contribute to HAND in individuals on ARVs (64).

Overall, HAND is the most widely accepted clinical manifestation observed in HIV-infected individuals that has been attributed to direct viral infection of myeloid cells. While it is impossible to discern between pathologies attributed to combinations of direct viral infection, interactions between viral proteins and brain-resident cells, and local inflammation, neurological disease is a very common event in HIV-infected individuals and novel therapeutic interventions to reduce or prevent it are greatly needed.

EVIDENCE THAT MACROPHAGES SUPPORT VIRAL REPLICATION

Several years after HIV was identified as the causative agent of AIDS, strains of HIV were described as either macrophage tropic ("M-tropic") or T cell tropic ("T-tropic"). For example, postmortem HIV isolates from the brain of a patient with AIDS dementia complex (HIV YU-2) and lung of a patient with AIDS (HIV Ba-L) were both identified as viruses that replicate efficiently in primary macrophages and were termed M-tropic (65, 66). Conversely, the first molecular clone of HIV, NL4-3, was obtained from the peripheral blood of a patient with AIDS, replicated in CD4 T cell lines but not non-T cell lines, and was identified as T-tropic (67). Further investigation demonstrated that the M-tropic and T-tropic nomenclature actually reflected the coreceptors utilized by each virus, with M-tropic and T-tropic viruses using CCR5 and CXCR4, respectively. A new viral nomenclature then emerged in which M-tropic viruses are R5 tropic and T-tropic viruses are X4 tropic (68). Though only a minority of CD4 T cells in the peripheral blood express CCR5, the majority of CD4 T cells within the GI tract express CCR5, and the vast majority of transmitted viruses are R5 tropic, with X4-tropic viruses generally emerging very late in infection (69–71).

R5 macrophage-tropic viruses tend to be defined by their ability to infect monocyte-derived macrophages (MDM) *in vitro* (72). In this system, monocytes are purified from peripheral blood and stimulated *in vitro* with lipopolysaccharide and IL-4, causing activation and differentiation into cells that resemble tissue-resident macrophages. At this point, the cells are significantly more prone to infection with HIV-1. Indeed, resting monocytes are very difficult to infect *in vitro* (70). That HIV-1 can very clearly infect MDM *in vitro* suggests that macrophages could support viral replication *in vivo*.

Importantly, though R5-tropic viruses comprise the majority of virus variants until late in infection, several studies have demonstrated that not all R5-tropic HIV-1 variants can infect macrophages and that factors beyond coreceptor dependence—including CD4-binding affinity and anatomic location—may influence what types of cells are infected (73). R5-tropic viruses that infect myeloid cells *in vitro* tend to have much higher affinity (up to 30-fold) for CD4 than viruses (R5 or X4 tropic) that cannot infect myeloid cells *in vitro* (74). The vast majority of CCR5-tropic HIV strains do not infect myeloid cells *in vitro* and might, instead, be classified as "R5 T cell tropic" (46, 48, 70, 74). Thus, a new nomenclature system has been suggested: R5 T cell tropic (the vast majority of viruses isolated from patients), R5 macrophage tropic (that evolve late in untreated infection to have a relatively higher affinity for CD4), and X4 T cell tropic (that evolve only late in infection) (70).

As previously stated, many tissue macrophages arise from differentiation of peripheral blood monocytes, and some data suggest that these monocytic macrophage precursors might be infected *in vivo*. Monocytes that circulate in peripheral blood are easily obtained from HIV-infected individuals. While these cells are extremely short-lived (either dying or differentiating into tissue macrophages [75]), some studies have suggested that viral DNA can be found within peripheral blood monocytes (76–79). Peripheral blood monocytes are separated into two subsets based upon expression patterns of CD16 and CD14, where $CD16^+$ monocytes are thought to have initiated differentiation into tissue macrophages (80). Monocytes that express CD16 are more prone to infection with HIV-1 *in vitro*, and some studies have found very low levels of viral DNA within these cells after flow cytometric sorting from peripheral blood of HIV-infected individuals (80, 81). However, other groups have reported that viral DNA is not detectable in peripheral blood monocytes from HIV-infected individuals (82, 83).

While peripheral blood is relatively easy to obtain from cohorts of HIV-infected and uninfected individuals, obtaining tissue-resident macrophages presents a particularly difficult problem. Two anatomical sites that have been surgically sampled in HIV-infected individuals are broncheoalveolar lavage (BAL) fluid and the intestine. Alveolar macrophages are the predominant cell type in BAL fluid. Thus, high numbers of alveolar macrophages can be obtained and isolated. Two studies have found that viral DNA can be detected in alveolar macrophages from HIV-infected individuals (84, 85). In both cases, alveolar macrophages from 70% of the subjects sampled contained viral DNA. Moreover, in each study, levels of viral DNA within alveolar macrophages were very low (even in treatment-naive, viremic, HIV-infected individuals), with around 1 in 100,000 alveolar macrophages containing viral DNA. Both studies also noted the difficulty in purely isolating alveolar macrophages given their autofluorescence and nonspecific antibody binding. Another study used flow cytometric sorting to isolate $CD4^+$ T cells and non-CD4 T cells from ileal and rectal biopsy specimens of HIV-infected individuals (86). In both anatomical sites the authors found low, but routinely detectable, levels of viral DNA in non-CD4 T cells, suggesting that myeloid cells were infected. However, other studies have suggested that GI tract-resident macrophages are resistant to HIV infection (87), and it is important to note that some HIV accessory proteins actively downregulate both CD3 and CD4 (88). Thus, CD4 T cells might become infected and then downregulate CD3 and CD4, making them appear as if they were not actually CD4 T cells. While these limited studies have routinely found viral DNA in tissue-resident macrophages from HIV-infected humans, all studies highlighted the need for further investigation given the limited numbers of cells that could be analyzed, the low levels of viral DNA observed, and the relative difficulty in studying and isolating

these cells that are rare, bind antibodies nonspecifically, and are autofluorescent.

ANIMAL MODELS OF MACROPHAGE INFECTION *IN VIVO*

The nonhuman primate SIV model of HIV infection has the potential to ameliorate at least one of the major limitations of research with human subjects. Namely, at necropsy, abundant tissues from any and all anatomical sites are available from nonhuman primates. Moreover, the availability of different strains of SIV, with differing macrophage tropism, allows for testing the hypothesis that tissue macrophages can support viral replication *in vivo*.

Early after HIV was described as the causative agent of AIDS in humans, researchers found a similar virus, SIV, that recapitulated HIV pathogenesis in Asian macaques (89). Since the discovery of SIV and its pathogenicity in nonhuman primates, there have been numerous identified or developed strains of SIV that cause very specific pathologies. A number of SIV models have been associated with significant viral replication in macrophages *in vivo*. SIVsmPBj is one of the first versions of SIV that was found to efficiently replicate in macrophage populations *in vivo* (90). When pigtail macaque monkeys are infected with SIVsmPBj, the animals very rapidly progress to death. Within a few weeks of infection, the animals experience severe diarrhea and generally require euthanasia to avoid death from dehydration. At the time of death, SIVsmPBj can easily be detected in GI tract lymphocytes, including macrophages, by *in situ* hybridization and immunohistochemistry. The disease caused by SIVsmPBj leads to a nearly complete destruction of the epithelial barrier of the GI tract.

The second SIV model wherein SIV efficiently replicates in macrophages *in vivo* is SIV/17EFr infection of pigtailed macaques (91). SIV/17EFr was grown from the brain of a SIV-infected animal that manifested neurological disease, and it is considered neurotropic. While the virus is not sufficient to cause disease in Asian macaques, when pigtail macaques are coinoculated with SIV/17EFr and a virus that replicates efficiently in memory CD4 T cells (such as SIV/ΔB670), the animals quickly (generally within 5 months) succumb to neurological disease. The ensuing neurological disease is associated with significant levels of virus in the CSF, and viral RNA can be detected by *in situ* hybridization within brain-resident cells.

Another immunodeficiency virus that infects macrophages *in vivo* is the HIV/SIV chimeric virus SHIVDH12 (92, 93). This virus was created molecularly by inserting a CXCR4-tropic envelope gene from an HIV molecular clone (HIV DH12) into the SIVmac239 clone (94). After inoculation and passage in rhesus macaques, a very pathogenic virus emerged that was subsequently subcloned and called SHIVDH12R. When SHIVDH12R is inoculated into rhesus macaques, the numbers of CD4 T cells in the animal drop precipitously, and after the CD4 T cell targets are depleted, viral replication can be detected in tissue-resident macrophages by *in situ* hybridization and immunohistochemistry (92, 93). Disease progression is rapid, with animals requiring euthanasia within 5 months postinfection, and viral RNA can be detected within macrophages from both lymphoid and GI tract tissues. That this HIV/SIV chimeric virus uses CXCR4 as a coreceptor and infects macrophages certainly challenges the idea that macrophage-tropic viruses require use of CCR5 as a coreceptor. Thus, alternative mechanisms for infection or interactions with macrophages *in vivo* that are independent of direct chemokine receptor-dependent infection may exist.

The three strains of viruses mentioned above (SIVsmPBj, SIV/17EFr, and SHIVDH12R) are not the common viruses that most researchers use in the field to study immunopathogenesis or to develop potential HIV vaccines. The most widely used SIV strains across the world are SIVmac251, SIVmac239, SIVsmE543, and SIVsmE660. SIVmac239 and SIVsmE543 are molecular clones, and SIVmac251 and SIVsmE660 are uncloned viruses and thus contain a swarm of individual viruses. Irrespective of the virus, infection route, and species of Asian macaque used, SIV-infected animals exhibit acute viremia that reduces to a set point, progressive loss of CD4 T cells from peripheral blood, and progression to simian AIDS in approximately 2 years. In rare instances, however, animals infected with one of these viruses rapidly progress to simian AIDS. These rapid progressor animals are fairly atypical in that they very quickly lose memory CD4 T cells and do not produce an antibody response to the virus (are virus seronegative). At the time of euthanasia, viral RNA can be detected in macrophages of multiple tissues by *in situ* hybridization and immunohistochemistry in these rapid progressors (95).

Among these four models of SIV that have been shown to efficiently replicate in macrophages, a common theme is the very rapid and nearly complete depletion of CD4 T cell targets. Massive depletion of CD4 T cells can also be accomplished experimentally with antibodies against CD4 that deplete CD4 T cells *in vivo*. Indeed, experimental depletion of CD4 T cells in rhesus macaques with depleting anti-CD4 antibody followed by infection with SIV leads to very rapid disease progression and robust viral replication within macrophages (96).

An additional area of investigation for infection of macrophages has focused on the role of viral accessory proteins in cellular targeting and disease progression. The viral protein X (Vpx) has been reported to promote viral replication in macrophages both *in vitro* and *in vivo* by targeting the host restriction factor SAMHD1 for proteasomal degradation (97–99). SAMHD1 inhibits viral replication in myeloid and dendritic cells by depleting the cellular pool of deoxyribonucleotide triphosphates for reverse transcription (99, 100). In HIV-2 and SIV strains of the SIVsm lineages, Vpx may facilitate efficient viral replication in macrophages by degrading SAMHD1. Indeed, in rhesus macaques infected with a *vpx* deletion mutation of SIVmac239, macrophages in the spleen and lymph node had a significantly lower frequency of SIV infection than animals infected with wild-type SIVmac239 (101). However, we recently reported a dramatic decrease in viral replication in rhesus macaques infected with SIVmac239ΔVpx but no increase in the frequency of viral DNA in the myeloid cell compartment compared to animals infected with wild-type SIVmac239 (102). In the same study, we saw no difference in the frequency of animals with viral DNA^+ myeloid cells in African green monkeys infected with SIVagm, which does not express Vpx. That tissue myeloid cells were not infected at a higher frequency in Vpx-encoding virus infection may be explained in part by recent reports that viral protein R (Vpr), an evolutionarily related viral accessory protein, may also degrade SAMHD1 in myeloid and dendritic cells (103, 104). Unlike Vpx, Vpr is encoded by all extant primate lentiviruses. Though additional characterization of the roles of Vpr and Vpx in HIV/SIV infection is needed, current data demonstrate the importance of considering differ-

ences between HIV and SIV strains when studying infection of macrophages both *in vitro* and *in vivo*.

In addition to the aforementioned Asian macaque SIV models, one group very recently developed a murine model for studying macrophage-tropic HIV *in vivo*. In this model, immunodeficient mice were reconstituted with fetal, human, hematopoietic cells that developed into myeloid cells but not T cells. These particular "humanized" mice, termed myeloid-only mice (MoM), could subsequently be infected with macrophage-tropic strains of HIV *in vivo* and support viral replication (83). Interestingly, very few CCR5-tropic viruses were able to replicate in MoM, suggesting this might be a very rare event *in vivo*.

Taken together, it is clear that primate immunodeficiency lentiviruses can have the capacity to replicate in myeloid cells *in vitro* and *in vivo*. However, it is important to note that the models wherein viral replication is routinely observed to occur within tissue-resident macrophages have two common themes: (i) massive systemic depletion of CD4 T cells and (ii) very rapid disease progression. Indeed, the four nonhuman primate models listed above are all associated with progression to AIDS in only a few months, compared to the "normal" progression rate of 1 to 2 years for SIVmac239, SIVmac251, SIVsmE543, and SIVsmE660 or compared to the "normal" progression rate of nearly 10 years for ARV-untreated HIV-infected individuals. Thus, though these models provide useful tools for studying viral replication in macrophages and pathologies that can occur in SIV-induced encephalitis, they do not necessarily provide conclusive information regarding the importance of SIV-macrophage interactions in the setting of normal progression in HIV-infected individuals, nor do these models necessarily suggest that macrophages represent a reservoir of infected cells after initiation of ARVs. Moreover, the SIV/Asian macaque animal model for HIV is further complicated by the finding that SIVs only very rarely evolve *in vivo* to use CXCR4 as a coreceptor, which might limit the appropriateness of this model for examination of cellular targeting by the virus in animals who have progressed to simian AIDS (105, 106).

METHODS FOR CHARACTERIZING HIV/SIV IN MACROPHAGES

Characterization of interactions between HIV/SIV and macrophages *in vivo* has relied, almost entirely, on detection of viral nucleic acids or viral antigens associated with these cells. This can be accomplished either by *in situ* hybridization, isolating macrophages from tissues followed by molecular detection of HIV/SIV RNA and/or DNA, or the use of antibodies against viral antigens (i.e., Gag protein) via immunohistochemistry. Some studies have, alternatively, used electron microscopy to identify the cellular localization of viral particles within macrophages.

In situ hybridization can be performed on formalin-fixed tissue sections. With this method, complementary oligonucleotide probes hybridize to the HIV/SIV genome and are revealed by microscopy. This analysis can be combined with monoclonal antibody staining against viral antigens and cellular antigens to identify which cells contain viral nucleic acids. Historically, *in situ* hybridization has relied upon the use of "riboprobes" to detect viral RNA where only cells containing multiple copies of viral RNA could be detected. This technology has become the "gold standard" for identifying cells that replicate HIV/SIV *in vivo*. Indeed, this analysis has been routinely used to identify viral RNA^+ myeloid cells in the macrophage-tropic SIV/nonhuman primate models listed above.

Examination of viral RNA^+ cells by *in situ* hybridization and viral antigen-positive cells by immunohistochemistry has been exhaustively performed using tissues from nonhuman primates infected with SIVmac239, SIVmac251, SIVsmE543, or SIVsmE660. Many of these studies have performed phenotypic analysis of virus-positive cells based upon expression (or lack thereof) of CD3, CD68, HAM56, and/or CD163 (107, 108). In SIV-infected macaques that rapidly progress, the majority of SIV viral RNA^+ cells appear to be macrophages (95). However, when chronically SIV-infected Asian macaques are examined, the vast majority of cells that are defined viral RNA^+ by *in situ* hybridization express CD3 (95). Cells that are virus positive but $CD3^-$ are assumed to belong to a myeloid lineage and can account for up to 10% of all virus-positive events in chronically infected animals (95). New technologies, termed DNAscope and RNAscope, have inherent signal amplifications that allow for detection of as few as one copy of viral nucleic acid (109). These techniques coupled with the cellular localization of the signal(s) may be able to identify cells with one integrated copy of viral DNA and could help to unambiguously identify the level to which macrophages become infected by the virus *in vivo*.

While *in situ* hybridization has been used to measure RNA-producing cells *in vivo*, alternative explanations exist to explain the detection of virus-positive cells by using this technique. One of the many functions that tissue-resident macrophages perform is clearance of dead and dying cells and of antibody/complement-coated immune complexes (26). Thus, macrophages that appear as viral RNA^+ by *in situ* hybridization might, occasionally, be in the process of clearing antibody/complement-coated HIV/SIV via phagocytosis. As mentioned previously, another approach to identify viral infectivity in individual subsets of cells *in vivo* involves disruption of tissues into single-cell suspensions with subsequent flow cytometric sorting of individual cells followed by PCR for viral DNA. Recently, we performed a fairly comprehensive analysis of viral DNA levels in CD4 T cell subsets and myeloid cells from multiple tissues of a large cohort of chronically SIV-infected Asian macaques (102). From this analysis, viral DNA was amplified from mucosal-resident macrophages from only two SIV-infected animals (colon-resident macrophages and jejunum-resident macrophages). Myeloid cells isolated from lymphoid tissues contained viral DNA in approximately 40% of the animals. These results are consistent with the anatomical localization of virus-positive cells in tissues where CD4 T cells are less dramatically depleted compared to mucosal tissues in progressively infected individuals (69). We therefore argued that routine viral acquisition by tissue-resident myeloid cells was dependent upon the presence of CD4 T cells in the same tissue.

Analysis of lymphoid tissues, particularly the lymph node, by *in situ* hybridization and immunohistochemistry has also shown that virus-positive myeloid cells are primarily found in tissues where CD4 T cells persist. In the lymph node, virus-positive $CD3^-$ myeloid cells are found almost exclusively within the paracortex area (V. M. Hirsch, unpublished data, and J. D. Estes, personal communication). One explanation consistent with this finding (viral DNA in myeloid cells in anatomical sites replete with CD4 T cells and the colocalization of viral RNA^+ cells in the lymph node paracortex) is phagocytosis of SIV-infected CD4 T cells by resident macrophages. Using quantitative PCR for rear-

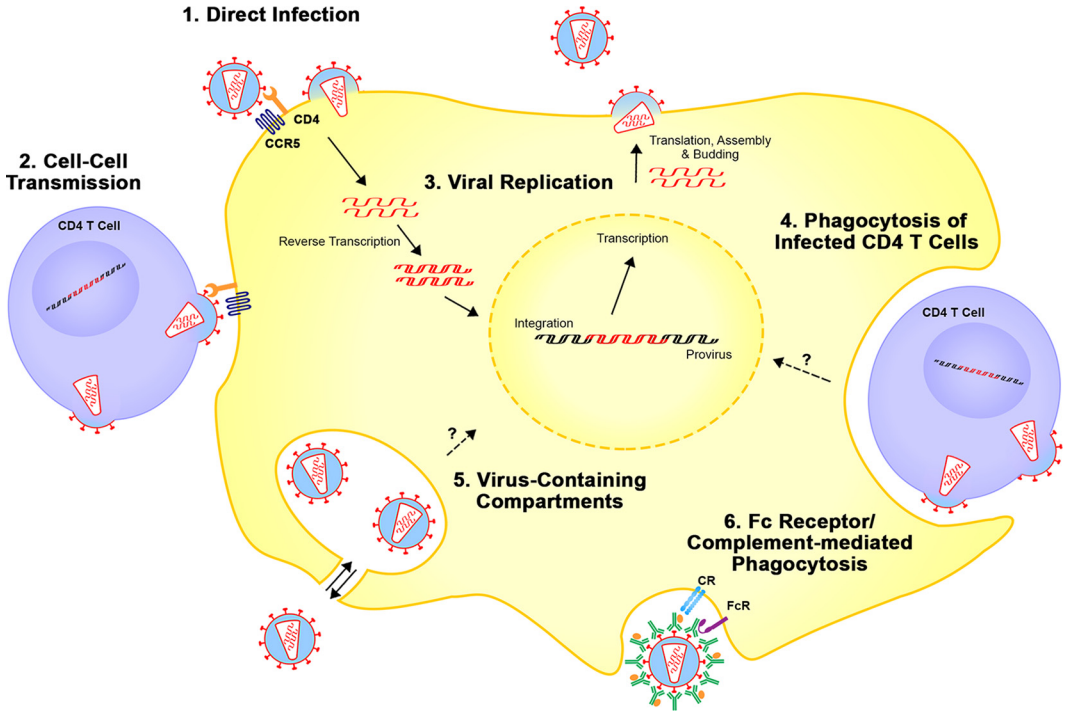

FIG 2 Reported interactions of macrophages with HIV/SIV. (1) Direct infection by cell-free virus via CD4 and CCR5; (2) infection of macrophages via cell-to-cell transmission; (3) viral replication in macrophages; (4) clearance of infected dead or dying CD4 T cells via phagocytosis; (5) compartmentalization of virions in virus-containing compartments; and (6) phagocytosis of antibody-coated virions via Fc receptors (FcR) or complement receptors (CR).

ranged T cell receptor genes, we showed that myeloid cells containing viral DNA also contained rearranged TCR DNA, suggesting that myeloid cells might have acquired the viral DNA by phagocytosis of SIV-infected CD4 T cells (102). While we were unable to determine whether or not the viral DNA we amplified from lymphoid tissue-resident myeloid cells represented replication-competent virus, it remains possible that virus within phagocytosed T cells could be responsible for latency and/or that macrophages become infected during the phagocytosis process. Indeed, recent work has suggested that HIV can replicate in macrophages after macrophage phagocytosis of HIV-infected T cells (110).

Consistent with the premise that viral replication can occur within myeloid cells through phagocytosis, analysis of HIV-infected macrophages via electron microscopy suggests that the virus is localized in membrane-bound intracellular compartments termed virus-containing compartments (VCCs) (111, 112). These VCCs closely resemble compartments formed during phagocytosis where lysosomes fuse with phagosomes before their contents are recycled through the cell into the extracellular space. Some have argued that viral replication in macrophages is physiologically different from that observed in T cells and that the virus replicates within these VCCs (111, 112).

CONCLUDING REMARKS

That macrophages are capable of supporting HIV/SIV replication is incontrovertible. Indeed, HIV can replicate in purified monocyte-derived macrophages *in vitro*, and there is compelling evidence that SIV replicates in macrophages *in vivo* when CD4 T cell targets are very dramatically depleted. However, it is unclear how macrophage infection contributes to the asymptomatic phase of HIV/SIV infection, and it is completely unclear whether or not macrophages represent a reservoir of replication-competent virus in ARV-treated, HIV/SIV-infected individuals. While it has been possible to isolate virus from anatomical sites where macrophages are presumed to be the source of virus *in vivo* (113), there have been no data generated to demonstrate that replication-competent virus can be retrieved from a macrophage population *ex vivo*. There are several important ways in which the virus can interact with macrophages and contribute to disease (Fig. 2). These interactions include (i) direct infection, (ii) viral replication, possibly via VCCs, (iii) phagocytosis of infected CD4 T cells, and (iv) phagocytosis of antibody/complement/virus complexes. How these interactions facilitate viral spread and/or disease progression remains unclear, and additional work is certainly warranted. Indeed, it remains critically important to determine the degree to which replication-competent virus can be retrieved from macrophages, whether this virus is genetically disparate from virus recovered from CD4 T cells, how currently used antiretroviral medications interfere with these processes, whether macrophages are a viral reservoir in ARV-treated individuals and, ultimately, if additional therapeutic interventions are required. Finally, expanded use of biopsy specimens or explants from both ARV-treated and untreated HIV-infected individuals would provide valuable information about the role of macrophages in HIV infection and the relevance of *in vitro* and animal models for studying the latent viral reservoir. Irrespective of the infection status of myeloid cells *in vivo*, their contribution to inflammation is indisputable and novel therapeutic agents to reverse myeloid cell activation *in vivo*, or therapies to reduce myeloid cell stimulatory factors, are greatly needed.

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