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INVITED REVIEW

Host and Viral Factors Influencing Interplay between the Macrophage and HIV-1

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Abstract

HIV-1 persists in cellular reservoirs that cannot be eliminated by antiretroviral therapy (ART). The major reservoir in infected individuals on effective ART is composed of resting memory CD4+ T cells that harbor proviral cDNA, and undergo a state of latency in which viral gene expression is minimal to absent. The CD4+ T cell reservoir has been extensively characterized. However, other HIV-1-permissive cells may contribute to HIV-1 persistence. Lentiviruses have a long recognized association with macrophages. However, the role, if any, played by macrophages in HIV-1 persistence is not well understood. Macrophages are resistant to cell death upon HIV-1 infection, and can survive for long periods of time, making them ideal host cells in which the virus might persist. Studying macrophages is challenging, as these cells reside in nearly all tissues. Moreover, detecting viral DNA or RNA in macrophages does not necessarily indicate that these cells will produce replication-competent viral particles. Currently, the gold standard assay to detect cellular reservoirs is the ex vivo quantitative viral outgrowth assay (QVOA), which requires a patient blood draw. However, macrophages reside deep within tissues that are inaccessible in living subjects, such as the central nervous system (CNS). Therefore, tools other than QVOA must be developed to identify cellular reservoirs that reside in the tissues. In this review, we will focus on the main aspects involved in HIV-1 persistence, including the molecular mechanisms of viral evasion, the main cell types responsible for harboring persistent HIV-1 and the tissue compartments that are likely to be reservoirs for HIV-1.

Keywords Macrophages · Persistence · CNS · Reservoir · HIV-1 infection

Introduction

The first weeks of HIV-1 infection, defined as the acute phase, is characterized by an exponential rise in viral load, rapid loss of CD4+ T cells, immune activation and symptoms such as headaches and fever. These conditions are ideal for explosive viral growth, which drives reservoir establishment very early after initial HIV-1 infection. However, the sooner an HIV-1-infected individual starts treatment with ART, the smaller the size of the reservoir, as seen by a delayed rebound when ART is interrupted (Li et al. 2016; Henrich et al. 2017).

HIV-1 infects two major cell types: CD4+ T cells (Barre-Sinoussi et al. 1983; Lifson et al. 1986; Maddon et al. 1986) and macrophages (Koenig et al. 1986). In terms of understanding viral persistence, most of the attention has focused on the resting memory CD4+ T cells; these cells are long-lived and harbor an integrated copy of HIV-1 cDNA. However, the size of HIV-1 reservoir in these cells is very small. Viral outgrowth assays have shown that approximately 1 in one million resting memory CD4+ T cells harbor replication-competent proviral HIV-1 DNA (Eriksson et al. 2013). From the total copies of proviral DNA that is detectable in resting memory CD4+ T cells, this small reservoir accounts for nearly 1% of the number of HIV-1 proviral DNA that is able to support the production of replication-competent virus. Moreover, this may not be the only reservoir responsible for sustaining persistent HIV-1. HIV-1 variants isolated at rebound viremia from ART-suppressed individuals, showed a considerable genetic discordance from plasma rebound HIV-1 compared to cell-

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associated RNA and replication-competent viruses found in latently infected memory CD4+ T cells prior to ART discontinuation (Chun et al. 2000). Although this study was performed in the 2000's, these cells are the only characterized reservoir for HIV-1.

In addition to CD4+ T-cells, macrophages are also permissive to infection by HIV-1 (Koenig et al. 1986). These cells are readily infected upon HIV-1 transmission, and do not show apparent signs of cytotoxicity following HIV-1 infection (Swingler et al. 2007; Coiras et al. 2009). Macrophages can be infected by HIV-1 and sustain a linear, steady HIV-1 production without undergoing cell death (Aquaro et al. 2002). Altogether, these features harbored by macrophages could likely contribute to viral persistence of the infected cell.

There is some debate regarding the mechanisms involved in reservoir maintenance. The prevailing view is that the reservoir is maintained by the intrinsic stability of the latently infected memory CD4+ T cell (Siliciano et al. 2003; Crooks et al. 2015). More recently, it has been shown that latently infected cells can undergo mitosis, which results in duplication of the latently infected cell (Chomont et al. 2009). While it was originally believed that this process predominantly leads to duplication of defective proviruses, it now appears that competent proviruses can be duplicated and serve as a template for the production of infectious viruses (Simonetti et al. 2016; Hosmane et al. 2017). It has also been shown that ART does not effectively penetrate all tissues to equal levels and that suboptimal drug concentrations might provide conditions for de novo infection (Fletcher et al. 2014). As a consequence, HIV-1-infected cells may not be completely eliminated by ART and may undergo a steady, "topping up" by homeostatic duplication and perhaps by generation of newly infected cells. HIV-1 replication in macrophages is also more resistant to inhibition by nucleoside analogue reverse transcriptase inhibitors (NRTI), as these require a phosphorylation step in order to become active, and phosphorylation activity in macrophages is reduced, compared to CD4+ T cells (Perno et al. 2006). In this review, we will examine the role of distinct types of macrophages in the context of HIV; the mechanisms imposed by the cellular immune response to eliminate the virus from infected cells, and will discuss the mechanisms by which macrophages may be potential candidates for HIV-1 persistence, including the most recent evidence supporting this hypothesis.

The Dynamics of HIV-1 Reservoir Maintenance and its Establishment in the Acute Phase

The first and only patient cured from HIV-1 reported to date, is Timothy Brown, known as the Berlin patient (Hutter et al. 2009). This patient developed acute myeloid leukemia after infection by HIV-1. He subsequently underwent 2 rounds of allogeneic hematopoietic stem cell transplantation (HSCT) with cells containing a homozygous Δ32 mutation in the gene encoding for CCR5, which conferred resistance to HIV-1 infection. The transplanted, new immune system replaced the old immune cells completely, and this patient remains in remission for over 8 years since he stopped ART (Hutter et al. 2009). The Berlin Patient has galvanized efforts amongst researchers to pursue a cure for HIV/AIDS using elements that led to the cure of Timothy Brown. As such, this remains an intense area of investigation. Some approaches have focused on early treatment initiation as a way to limit reservoir size and thereby make the task of eliminating the reservoir less challenging. Li and colleagues have shown that the earlier an HIV-1-infected individual starts treatment, the smaller is the size of the reservoir. Therefore, individuals who initiated ART during acute HIV-1 infection, exhibited delayed virus rebound following treatment interruption, and this was associated with low levels of cell associated-RNA (CA-RNA) present pre-ATI. However, those with higher pre-ATI CA-RNA, showed shorter time of rebound and had significantly higher levels of residual viremia pre-ATI (Li et al. 2016).

Another recent study evaluated whether initiating ART extremely early during acute HIV-1 infection would prevent HIV-1 from establishing a reservoir and ultimately prevents rebound. The study involved two HIV-1 infected patients who were on pre-exposure prophylaxis (PreP) program after 10–12 days post-infection (Henrich et al. 2017). Patient A had very low plasma viremia before ART initiation. The participant achieved undetectable viral load, and after extensively sampling failed to detect HIV-1 from several distinct tissue sites over the course of 2 years, ART was stopped. However, 7.4 months later, HIV-1 rebounded. This case resembles the case of one of the Boston patients (patient B), who underwent allogeneic bone-marrow transplantation, followed by 3 years on ART (Henrich et al. 2014). Upon treatment interruption, the patient remained on remission for 8 months prior to HIV-1 rebound. These two cases suggest that, although preventive measures were taken to reduce viral reservoir size to undetectable levels, ultimately, HIV-1 still persisted. This has taught us that the inability to detect HIV-1 does not mean it is not there.

Overview of Lentivirus Infection in Macrophages

Infection of a cell first requires interaction of the viral envelope glycoprotein with CD4, which is the primary receptor for HIV-1. A conformational change in the viral envelope that is created by CD4 interaction then exposes co-receptor binding epitopes on the viral envelope. While infection of CD4+ T cells is readily achieved by HIV-1, CD4 expression on macrophages is very low - barely detectable by FACS – such that viruses capable of infecting macrophages require an envelope

with a high affinity for CD4. This is the primary determinant of macrophage tropism (Kumar et al. 2014). Initial infection is initiated by so-called transmitted/founder (T/F) variants. These viruses usually infect cells expressing high levels of the CD4 receptor on the cell surface but are unable to infect macrophages (Salazar-Gonzalez et al. 2009). Therefore, during the course of infection, HIV-1 must undergo changes in envelope that confers high CD4 affinity and macrophage tropism. However, the mechanisms by which HIV-1 has evolved to efficiently infect these cells remain unclear. The rapid depletion of CD4+ T cells in the acute phase of infection may favor the appearance of genetic HIV-1 variants that undergo evolutionary pressure to infect other cell types. However, upon early ART initiation, T/F HIV-1 strains may not undergo enough genetic diversification to allow for rapid evolution and subsequent appearance of macrophage-tropic variants.

Macrophages are cells of the innate immune system that serve as scavengers to eliminate cells infected by pathogens. Macrophages are found in tissues, and are responsible for protecting these sites from infection. Therefore, they can be found in nearly every tissue in the body and may have distinct functions depending on their location. For instance, the expression of key receptors necessary for HIV-1 infection may vary according to the site where the macrophage reside in, which determines the susceptibility to HIV-1 among different macrophage phenotypes. Shen and colleagues demonstrated that vaginal macrophages, in comparison to gut macrophages, are more susceptible to HIV-1 infection and are able to sustain HIV-1 replication, as they express the key receptors for HIV-1 entry including CD4 and CCR5 (Shen et al. 2009). In other tissues, the presence of HIV-1 DNA and RNA has been detected in alveolar macrophages of HIV-1-infected individuals on effective ART, suggesting the potential of these cells to harbor persistent virus (Jambo et al. 2014; Cribbs et al. 2015). Moreover, alveolar macrophages harboring proviral HIV-1 DNA have been associated with a reduced phagocytic activity, leading to a higher risk of acquiring respiratory infections.

Although less studied, Kupffer cells, the resident tissue macrophage in the liver, have been also reported to undergo infection by HIV-1 (Schmitt et al. 1990; Hufert et al. 1993; Mosoian et al. 2017). An early study analyzed 7 *post-mortem* liver tissues of patients who developed AIDS. HIV-1 DNA was found in the liver, as well as in peripheral blood monocytes in 3 out of 7 patients analyzed (Hufert et al. 1993). More recently, a study demonstrated that when Kupffer cells are cultured ex-vivo, followed by exposure to HIV-1, these cells become productively infected. They have also shown an enhancement of CD14 and TLR4 expression in these cells (Mosoian et al. 2017), thereby promoting pro-inflammatory responses that may impact negatively the immune response to fight against hepatic inflammation and bacterial translocation.

The least studied type of macrophages, but perhaps the most important in terms of HIV-1 pathogenesis, are the brain macrophages, including the microglia and perivascular macrophages. Infection of these cells is associated with HIV-1 pathogenesis and its complications throughout the course of HIV-1 infection and development into AIDS. However, in HIV-1-infected individuals, there are very limited studies associating the HIV-1 reservoir to human microglia, as this tissue is not readily accessible in vivo. Some early studies from the 80's have demonstrated the presence of HIV-1 infection in the CNS, and these studies suggested the presence of HIV-1 in myeloid cells in the brain. HIV-1 was detected in post-mortem brain tissue of individuals who have developed AIDS by in-situ hybridization, mainly in the white matter, and restricted to endothelial capillaries, mononuclear inflammatory cells and giant cells (Wiley et al. 1986). Another study performed in sections of the brain, demonstrated the presence of HIV-1 (formally HTLV-III) in cells that resembled monocyte/macrophages, and were localized in the cortex of the frontal, temporal, and parietal lobes (Gabuzda et al. 1986). Another study demonstrated the presence of mononucleated and multinucleated macrophages that were actively producing viral RNA and viral particles in the brain of AIDS patients (Koenig et al. 1986). Although these studies demonstrated that myeloid cells in the brain are able to support HIV-1 infection, it is unclear whether these cells harbor persistent HIV-1 in patients on suppressive ART that could generate productive viral particles once ART is interrupted. More recently, studies have demonstrated the presence of infected microglia and peripheral macrophages in the brain of HIV-1-infected individuals on suppressive ART. Post-mortem brain biopsies of two individuals on effective ART were assessed for the presence of viral RNA, total and integrated HIV-1 DNA, which were readily detected (Asahchop et al. 2017). In this same study, they found that concentration of several antiretroviral drugs was substantially reduced in different regions of the brain, which may contribute to viral persistence, even in individuals on suppressive ART.

Despite the inaccessibility of this tissue, HIV-1 has been found in microglia and perivascular macrophages in the CNS of HIV-1-infected adults from adult cultured temporal lobe tissue (Albright et al. 2000) and from brain autopsy tissue in infants (Ioannidis et al. 1995). Other studies have taken advantage of studying the cerebrospinal fluid (CSF) to predict the dynamics of HIV-1 infection in the brain (Schnell et al. 2011). Despite efforts to reach out to this tissue, the CNS remains one of the least studied sites in the body, due to the difficulty in accessing tissue sites. Therefore, it remains unclear whether the CNS, including microglia, is a truly reservoir for HIV-1.

Macrophages not only play an important role in HIV-1 infection, but also are critical in the context of lentiviral infections across several other animal species. These cells are permissive to a wide range of lentiviral infections. In goats, the

caprine arthritis-encephalitis virus (CAEV) infects blood-derived caprine monocytes, with increased infectivity when differentiating into macrophages (Anderson et al. 1983). The infection of caprine macrophages by CAEV is also suggested to play a role in viral persistence in these cells. Another lentivirus found in sheep, the visna virus is characterized by a slow disease progression, and long periods of incubation (Narayan 1983). Early studies have shown that the visna virus infects macrophages in the lung, as observed by the presence of viral RNA in these cells. However, the presence of RNA was not correlated with viral replication. The study found macrophage precursor cells in the bone marrow harboring replicate-competent visna virus, suggesting a reservoir of latently infected cells (Gendelman et al. 1985).

The CNS as a Reservoir for HIV-1

Most of the information regarding the association of HIV-1 with the CNS has been derived from a plethora of pathologic, immune, behavioral and virologic studies (Moskowitz et al. 1984; Levy et al. 1986). These studies have shown that the CNS is susceptible to infection in immune-compromised HIV-1 individuals. Infection of microglia in the CNS is also believed to underscore HIV-1 neuropathogenicity (Persidsky and Poluektova 2006; Wachter et al. 2016). In SIV/macaque model, brain macrophages and microglia are also permissive to infection (Micci et al. 2014). Prior to the advent of ART, the incidence of HIV-associated dementia (HAD) was over 50% in HIV-1-infected individuals. However, even after ART era, there have been several cases of neurocognitive impairment that vary in severity among ART-treated individuals (Antinori et al. 2007). Of the individuals on ART, a considerably percentage of HIV-1-infected subjects still manifests neurological symptoms, that associated with an increase in makers for neuronal inflammation and a decrease in a marker for neuronal integrity (Harezlak et al. 2011). HIV-1 is a neurotropic virus that is believed to cross the blood brain barrier (BBB) through a mechanism known as "Trojan horse" (Kramer-Hammerle et al. 2005). Circulating cell-free virus, and HIV-1 infected monocytes and CD4+ T cells are recruited to the CNS through cytokine secretion by brain endothelial cells upon the presence of viral proteins (Fig. 1) (Albright et al. 2003). Infected cells and free-cell virus cross the BBB through transcytosis of the endothelial cells, into the brain. Cell-free viruses also cross the BBB through damaged tight junctions, to further infect astrocytes and microglia. Infection of cells in the CNS leads to a cascade of events, with release of proinflammatory cytokines as IL-1β, TNF-α, and HIV-1 viral proteins Tat and gp120 (Fig. 1), which contribute to neuronal dysfunction (Li et al. 2011; Mamik et al. 2011). HIV-1-infected individuals may consequently experience cognitive, behavioral, and motor dysfunction due to consequences of HIV-1-associated CNS damage. This series of cyclical events leads to loss of

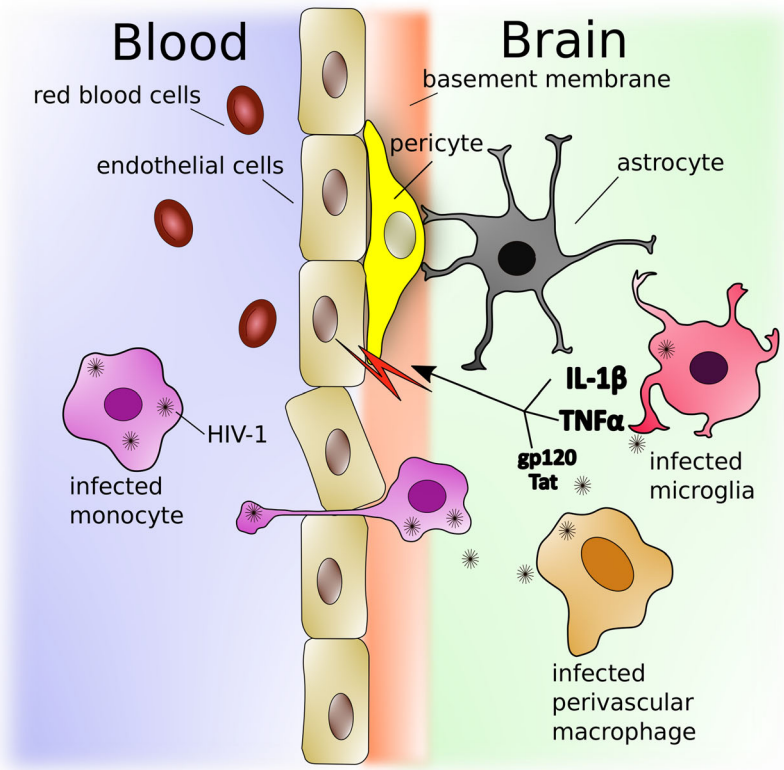

Fig. 1 HIV-1 crosses the blood brain barrier by a "Trojan horse" mechanism

pericytes, which are supportive cells of the endothelial vasculature. *Post-mortem* analysis of the brain of HAD subjects show that an increased number of monocytes are found in the perivascular space of the CNS (Fischer-Smith et al. 2001). In a mouse model, monocytes exposed to proinflammatory molecules, such as LPS and TNF-α, and IL-10, exhibited greater CNS migration, as compared to non-activated monocytes (Persidsky et al. 1997). This likely contributes to the replenishment of perivascular macrophages, which could in turn contribute to an expansion of the HIV-1 reservoir in the CNS.

Infected cells, primarily monocytes, are recruited to the CNS site through cytokine secretion by the brain endothelial cells in the presence of viral HIV-1 proteins. HIV-1 is then released into the CNS to further infect microglia and perivascular macrophages. Infection of brain myeloid cells further stimulates cytokine production by infected microglia and macrophages *in trans*. IL-1β, TNF-α and the HIV-1 proteins gp120 and Tat activates the brain barrier endothelial cells and pericytes, leading to inflammation and increased expression of tight junction molecules. This series of events leads to decreased pericyte function, which may induce changes in BBB permeability, to allow the recruitment of additional infected monocytes and cell-free viruses to the CNS.

Because the BBB is a highly controlled barrier to protect the CNS, some antiretroviral drugs may access CNS tissues at sub-optimal concentrations, which may further undermine HIV-1 control in microglia and perivascular macrophages. Since myeloid-derived cells are the main cell population infected by HIV-1 in the brain, and since the CNS may serve as a drug sanctuary for HIV-1, this may create conditions for viral persistence. This is even more evident considering that different levels of HAD can be manifested in ART-treated patients, indicating that brain damage still occurs, despite antiretroviral treatment.

Obstacles to Studying HIV-1 Persistence in Macrophages

The study of HIV-1 in human macrophages from living subjects is a daunting experimental undertaking. Macrophages reside in nearly all tissues in the human body, some which are inaccessible in living subjects. There is the issue of sampling in that a biopsy of lymph nodes may not be representative of what is happening in specialized macrophages in other tissues. In addition, some tissues are inaccessible in living subjects. This includes the CNS, which is one of the most important sites in terms of HIV-1 infection of macrophages. As mentioned above, studies from the SIV/macaque and humanized models predict that microglia and perivascular macrophages are likely to be infected by HIV-1, which could contribute to HIV-1 persistence.

It has been argued that the presence of SIV DNA in macrophages from SIV-infected macaques may be from a phagocytized T cell rather than through active infection of macrophages (Calantone et al. 2014). However, phagocytosis of infected CD4+ T cells could represent a novel mechanism for myeloid cell infection (Baxter et al. 2014). Furthermore, in the SIV/macaque model, macrophage infection is not observed with viral variants that have envelopes that are strictly T cell tropic (Bannert et al. 2000). In other words, T cell phagocytosis of infected CD4+ T cells may occur but appears to be rare.

Virus-Host Interplay: The Cell Mediated Immune Response to HIV-1 Vs the Efforts of HIV-1 to Evade it.

During the course of infection, HIV-1 overcomes the effects of the immune system through viral proteins that target cellular responses against the virus. Once infected, macrophages release inflammatory cytokines and chemokines that recruit other cells of the innate and adaptive immune system (Xing et al. 2009; Ben Haij et al. 2015). Although the innate immune system efficiently mounts an antiviral response against HIV-1, viral proteins are able to overcome the antiviral effects of the immune system through several mechanisms. The HIV-1 accessory proteins play a central role in the viruses evasion of host antiviral immunity.

A common feature amongst different pathogens is there ability to evade the host's immune response by down regulating major histocompatibility complexes molecules (MHCs), thereby preventing recognition by cytotoxic T lymphocytes (CTLs). The accessory protein, negative factor (Nef), down regulates MHC-1, which prevents macrophages and T lymphocytes from presenting HIV-1 antigens to CD8+ T cells. There are two proposed models by which Nef downregulates MHC-1 (Fig. 2); the first is by binding to MHC-1 in the endoplasmic reticulum (Atkins et al. 2008; Dikeakos et al. 2010), followed by migration to the *trans*-golgi network (TGN) (Fig. 2A). Nef-MHC-1 complex is then phosphorylated, preventing trafficking to the cell membrane and consequently transported to lysosome for degradation (Kasper et al. 2005). The second is through increasing endocytosis of cellular MHC-1, by transporting into endosomes followed by lysosomal degradation (Fig. 2B) (Atkins et al. 2008).

Nef downregulates MHC-1 on the cell surface thereby preventing antigen presentation to cytotoxic CD8+ T cells. There are two proposed mechanisms by which Nef disrupts MHC-1. (A) The first is by binding of Nef to MHC-1 in the endoplasmic reticulum, followed by migration to the *trans*-Golgi network (TGN). Upon MHC-1 phosphorylation, Nef-MHC-1 complex is diverted from the TGN and sequestered to lysosomal compartments, where MHC-1 will be degraded.

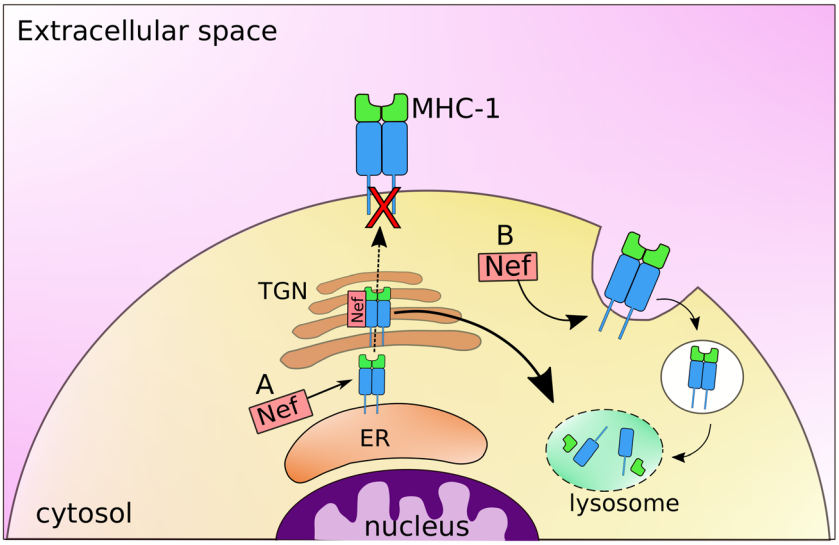

Fig. 2 Mechanisms of viral evasion regulated by HIV-1 protein Nef

(B) The second mechanism, by which Nef downregulates MHC-1, is through increased endocytosis of cellular MHC-1, followed by lysosomal degradation.

Another function attributed to Nef, in association with the viral protein Vpu, is the downregulation of an important receptor associated with leukocyte turnover, L-selectin (CD62L) leukocyte homing receptor. CD62L is associated with cell transcytosis on the vascular endothelium of peripheral lymph nodes. Upon infection of primary CD4+ T cells, levels of CD62L on the cell surface are reduced due to sequestering of CD62L in perinuclear compartments, triggered by Nef and Vpu (Vassena et al. 2015). By blocking CD62L functions, leukocytes are prevented from eliminating pathogens, allowing HIV-1 to evade the host immune response.

In addition to cell-mediated immunity, there are host proteins that potently antagonize HIV-1 infection and here too, the viral accessory proteins play a key role in circumventing these obstacles. SAM domain and HD domain-containing protein 1 (SAMHD1) reduces dNTP concentrations in non-mitotic cells such as macrophages where dNTPs are excessive to need. Since dNTPs are also required for reverse transcription of viral DNA, infection of macrophages is limited in the presence of SAMHD1. For HIV-2 and SIV, the situation is clear. The viral Vpx protein interacts with SAMHD1 and targets it for proteasomal destruction thereby raising dNTP concentrations to levels conducive to efficient reverse transcription. However, HIV-1 does not harbor a Vpx protein and the related Vpr protein does not appear to harbor a Vpx-like activity (Sharova et al. 2008). Some studies suggest that HIV-1 has evolved a reverse transcriptase that can polymerize in low dNTP environments (Lenzi et al. 2015; Nguyen et al. 2015). Furthermore, some studies have proposed that macrophages in G1-like stage have reduced levels of SAMHD1 (Kyei et al. 2015; Mlcochova et al. 2017). When macrophages enter a G1-like stage, cycling-dependent kinases (CDKs) phosphorylate SAMHD1, reducing its activity. Collectively, this suggests that HIV-1 has been hard pressed to come up with a way to avoid SAMHD1 restriction and argues that infection of macrophages might serve an important role in virus-host interplay.

Macrophages are also resistant to cell killing by CD8+ T lymphocytes. In a study using a SIV/macaque model, SIV-specific CD8+ T cells were freshly sorted and co-cultured ex-vivo with SIV-infected autologous CD4+ T cells or monocyte-derived macrophages from naïve macaques. The study found that viral replication was suppressed in infected CD4+ T cells, whereas CTLs failed to eliminate infected macrophages (Vojnov et al. 2012; Rainho et al. 2015). This study suggests that macrophages are resistant to CD8+ T cell killing in primate models, and therefore may be contributing to viral persistence in primates.

Why would Macrophages Be a Suitable Candidate for a Viral Reservoir?

There are several lines of evidence supporting the notion that macrophages are reservoir for HIV-1. However, for a cell to be considered reservoir, it must harbor a proviral DNA that upon reactivation, is capable of generating replication competent viruses. There have been studies in animal models showing that macrophages are a viral reservoir (Arainga et al. 2017; Avalos et al. 2017; Honeycutt et al. 2017). However, the same conclusion cannot be made for those HIV-1-infected individuals suppressed by ART.

Perhaps the first major evidence that macrophages are potential reservoirs for HIV-1 came from a study in macaques, in which CD4+ T cells were gradually depleted followed infection by a highly pathogenic SIV/HIV-1 chimera (SHIV) strain. In the absence of CD4+ T cells, high levels of plasma viral RNA were maintained that was found to originate from tissue

macrophages (Igarashi et al. 2001). They also demonstrated that infected macrophages were resistant to a reverse transcriptase inhibitor, as compared to infected CD4+ T cells, suggesting that macrophages may persist in the absence of continuous rounds of infection.

Some studies have shown compartmentalization of distinct viral variants in the CNS. For example, HIV-1 infected individuals with symptoms of HIV-associated dementia demonstrated the presence of two distinct HIV-producing cells as seen by the genetically divergent envelope (*env)* sequences associated with experimentally defined phenotypes. In HAD individuals, they observed a rapid decay in T-cell tropic (T-tropic) viruses in the CSF, which correlated with the decay seen in the blood. In contrast, macrophage-tropic (M-tropic) viruses exhibited a slow decay in the CSF after ART initiation, indicating a long-lived reservoir of virus producing cells. Parallel to this study, a model of SIV in macaque also confirmed the presence of compartmentalized SIV variants that evolved independently of viruses found in the periphery, indicating a complete independent evolution and tissue compartmentalization in the brain (Gama et al. 2017). Despite ART, neurological disorders of wide magnitude is found in HIV-1 infected individuals, demonstrating the relevance of HIV-1 infection in resident cells in the CNS (Bhaskaran et al. 2008; Jevtovic et al. 2009).

There have been several studies using humanized mouse models to study HIV-1. In a particular study, macrophages have been shown to harbor HIV-1 RNA and DNA even in mice suppressed by ART (Arainga et al. 2017). In a humanized myeloid only mouse (MoM) model in which human macrophages but not CD4+ T cells are reconstituted, replication-competent HIV-1 was found in several tissues, including brain, confirming that, at least in this model, macrophages sustain HIV-1 infection independently of CD4+ T-cells (Honeycutt et al. 2016). More recently, the same group took advantage of the MoM model, to evaluate whether macrophages can also harbor persistent HIV-1 in ART-suppressed MoM. In this study, they showed that HIV-1 could be recovered following treatment interruption. HIV-1 rebounded in approximately 33% of mice at the time of HIV-1 rebound (Honeycutt et al. 2017). The study concluded that in MoM, macrophages were capable of harboring latent HIV-1 and that upon treatment interruption, these cells produced replication competent viruses. While this study is the first to demonstrate a macrophage reservoir in vivo, it is unclear whether the lifespan of macrophages reconstituted in the MoM model were comparable to the lifespan of brain macrophages. In addition, the absence of CD4 T cells limits interpretation of the model and its application to the understanding of HIV-1 infection in humans. Nevertheless, the study provides support to the hypothesis that macrophages serve as a viral reservoir under effective ART.

Macrophages have also been shown to harbor replication competent virus in ART-suppressed macaque (Avalos et al. 2017). In the study, the investigators took advantage of a modified QVOA that was adapted for macrophages, in which brain macrophages were isolated and cultured ex vivo with feeder cells and a mix of molecules such as TNF-α and prostaglandin to promote cell activation. The investigators found that in approximately 87% of ART-suppressed macaques, replication-competent virus was rescued from cultures of macrophages (Avalos et al. 2017).

Establishment of latency in macrophages has also been thought to play a distinct role from CD4+ T-cells. Although the mechanisms by which CD4+ T-cell establish latency are well known, more investigations are needed to fully understand the mechanism in macrophages. At least for CD4+ T-cells, there are two described mechanisms of latency; the first, established in naïve T-cells that carry HIV-1 unintegrated DNA, is the pre-integration complex (Zack et al. 1990). This form of latency is characterized by poor reverse transcriptase activity, which impairs synthesis of proviral DNA (Bukrinsky et al. 1992). However, the clinic relevance of this form of latency is debatable, as naïve T-cells in pre-integration latency are short-lived (Pierson et al. 2002), and therefore may not contribute to long-term HIV-1 persistence. In the contrary, macrophages support unintegrated HIV-1 DNA transcription for long periods of time (Kelly et al. 2008), thus suggesting a role in HIV-1 latency.

The second form of latency, the post-integration of HIV-1 proviral DNA into the host's genome, plays an important role for long-term latency. There are two proposed mechanisms; the first suggests that HIV-1 proviral DNA integrates into heterochromatin region, and therefore is not expressed. The second is the integration into euchromatin regions, which might or might not be expressed. That will depend on many factors, such as the integration site, and the promoters and molecules involved in regulating cellular transcription. The HIV-1 Tat protein plays a crucial role for production of new viral particles. Its functions include interaction with the cellular protein kinase activity TAK (Tat-associated kinase)/P-TEFb (Mancebo et al. 1997, (Zhu et al. 1997), composed of CDK9 and cyclin T1, for its transactivation function (Wei et al. 1998). This step in HIV-1 infection may be different in T-cells and macrophages, and could contribute to distinct mechanisms of latency. TAK/P-TEFb activity is lower in resting CD4+ T-cells, which may be limiting HIV-1 replication. Levels of CDK9 and T1 mRNA and protein are increased upon T-cell activation, consequently inducing TAK/P-TEFb kinase activity, with increased viral replication (Garriga et al. 1998; Herrmann et al. 1998). On the other hand, in circulating monocytes, CDK9 protein levels are elevated (Dong et al. 2009), while cyclin T1 protein levels are close to undetectable. In early-differentiated macrophages, post-transcriptional regulation mechanisms lead to an increase in cyclin T1 protein

expression. Later during differentiation, expression of cyclin T1 starts to decrease, leading to a reduction in Tat transactivation (Liou et al. 2004). However, upon inflammatory stimuli, such as LPS, cyclin T1 can be re-induced in macrophages. Altogether, these studies show that latency mechanisms in macrophages may be different from those in CD4+ T-cells, though there may be other factors and pathways contributing to latency in macrophages that are unknown. To achieve a sterile cure, more research is needed to address the mechanisms of latency in cell populations that act as reservoirs.

Conclusions

HIV-1 is a leading cause of morbidity and mortality worldwide. Although ART has dramatically impacted the outlook for individuals living with HIV-1 infection, the majority of infected individuals do not have access to therapy. The use of ART in prevention is also limited and only a fraction of susceptible individuals at risk of infection are on PrEP (King et al. 2014; McMahon et al. 2014). Even in resource- rich countries, lifetime ART is not a solution. Antiretroviral resistance leads to high rates of failure (Fogel et al. 2016; Tsai et al. 2017). Every year, one million individuals die from AIDS-related diseases. Collectively, these facts argue for the development of a cure for infection. Key to this task is a thorough understanding of the viral reservoirs that sustain HIV-1 persistence in the face of suppressive ART. This information would then aid in the design of strategies to eliminate the reservoirs in which the virus persists. Most of the attention has focused on the latent CD4+ T cell. Strategies for elimination of latently infected cells are being explored in the clinic. Those approaches have centered on reactivating the latently infected cell with the hope that renewed viral antigen production would result in clearance of the infected cell by cell-mediated immunity and/or viral cytopathicity. However, those approaches may have limited effectiveness against the macrophage reservoir where the effects of cell mediated immunity and viral cytopathicity are less apparent. Basic questions still need to be answered. Does HIV-1 reside in a latent state in macrophages? If so, what agents might be used to reactivate latent infection of macrophages? What is the longevity of an infected macrophage? Answers to those questions, challenging as they are, would drive a concerted effort to develop approaches for elimination of the macrophage reservoir. It would be better to conduct those studies in concert with studies on the CD4 T+ cell reservoir. Otherwise, we may ultimately discover that removal of the reservoir of CD4+ T cells is not sufficient to cure HIV-1 infection.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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