Review

Eradication of HIV-1 from the Macrophage Reservoir: An Uncertain Goal?

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Abstract: Human immunodeficiency virus type 1 (HIV-1) establishes latency in resting memory CD4+ T cells and cells of myeloid lineage. In contrast to the T cells, cells of myeloid lineage are resistant to the HIV-1 induced cytopathic effect. Cells of myeloid lineage including macrophages are present in anatomical sanctuaries making them a difficult drug target. In addition, the long life span of macrophages as compared to the CD4+ T cells make them important viral reservoirs in infected individuals especially in the late stage of viral infection where CD4+ T cells are largely depleted. In the past decade, HIV-1 persistence in resting CD4+ T cells has gained considerable attention. It is currently believed that rebound viremia following cessation of combination anti-retroviral therapy (cART) originates from this source. However, the clinical relevance of this reservoir has been questioned. It is suggested that the resting CD4+ T cells are only one source of residual viremia and other viral reservoirs such as tissue macrophages should be seriously considered. In the present review we will discuss how macrophages contribute to the development of
long-lived latent reservoirs and how macrophages can be used as a therapeutic target in eradicating latent reservoir.

**Keywords:** HIV-1; cART; latency; reservoirs; macrophage

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1. Introduction

More than 35 million people have been infected with human immunodeficiency virus type-1 (HIV-1) worldwide [1,2]. With the introduction of combination anti-retroviral therapy (cART) in 1996 HIV-1 infection has become treatable but yet not curable [3–7]. Today, more than 30 different antiretroviral drugs have been approved for HIV treatment [2,8]. These drugs drive the viral load down to undetectable levels. However, the persistence of latent reservoirs of replication-competent non-induced proviruses remains a major obstacle in HIV-1 eradication [3,9–16]. These latent reservoirs are established early during acute viral infection [17–19]. Macrophages and latently infected resting CD4+ T cells are reservoirs of HIV-1 [20–23]. These reservoirs are fully capable of producing infectious viral particles when cART is discontinued [11,15,19,24].

Based on the integration status of HIV-1 proviral DNA into the host chromatin, latency has been classified as pre and post integration latency [25–28]. The role of unintegrated forms of HIV-1 DNA in the formation of viral reservoir is not well established. However, tissue specific cells retain these forms for a longer period of time [29,30]. Post-integration latency occurs when a provirus fails to adequately express its genome and becomes reversibly silenced after integration into the host genome. This latent state is exceptionally stable and mechanisms that maintain HIV-1 latency in vivo are not fully understood. Several factors contribute to the silencing of integrated HIV-1 provirus such as the site and orientation of integration into the host genome. These factors include the absence of crucial inducible host factors, the presence of transcriptional repressors, the chromatin structure and epigenetic control of HIV-1 promoter, sequestration of cellular positive transcription factors and the suboptimal concentration of viral transactivators, and inhibition of HIV-1 translation by microRNAs [15,31–36]. Most of these mechanisms have been elucidated using transformed cell lines and recently developed primary cell models of HIV-1 latency. However, the relative importance of each mechanism in maintaining viral latency in vivo is not fully established.

Reports suggest the HIV-1 infection of circulating monocytes in vivo. The infected monocytes can cross the blood-tissue barrier and can differentiate into macrophages [18,26,37–39]. Moreover, HIV-1 infected macrophages release several immunoregulatory and inflammatory cytokines including TNF-α, interleukin (IL)-1, and IL-7, which in turn influence viral replication and disease associated with viral infection [40,41]. The successful blockade of HIV-1 replication by cART has shifted the medical research from developing novel antiretroviral drugs towards the eradication of viral reservoirs. A better understanding in the formation of HIV-1 reservoirs will be necessary to uncover the novel targets and methods for purging or eradicating the latent reservoirs. The purpose of this review is to precisely define the viral reservoirs for therapeutic applications.
2. HIV-1 Infection of Monocytes/Macrophages

Macrophages play a crucial role in the initial infection, and contribute to HIV-1 pathogenesis throughout the course of viral infection. Since macrophages are an important part of innate immunity and participate indirectly to the adaptive immunity to clear the infection, this makes them a central target of HIV-1 [37,42–50]. HIV-1 targets the monocyte/macrophage lineage at varying stages of differentiation [48,49]. For instance data suggests the involvement of a particular monocyte subtype in HIV-1 infection [51]. Phenotypical comparative studies demonstrate that CD14+CD16+ monocytes are more permissive to productive HIV-1 infection and harbor HIV-1 in infected individuals on cART as compared to the majority of blood monocytes (CD14++CD16−). In healthy individuals, the CD14++CD16- monocytes represent 10% of circulating monocytes [52]. The characteristics have been studied in rhesus macaques. In acute infection, there was an increase in CD14++CD16+ and CD14+CD16− monocytes, while CD14++CD16+ monocytes decreased two weeks after infection [53]. Similarly, there was an increase in CD14++CD16+ and CD14+CD16− monocytes subsets in rhesus macaques with chronic infection and high viral load [53,54]. Moreover, in HIV-1 infected patients, the preferential expansion of CD14++CD16+ monocyte subset is associated with increased intracellular level of CCL2 [55]. CCL-2 is an important pro-inflammatory chemokine produced during HIV-1 infection and is one of the key factors responsible for the chronic inflammation and tissue damage in HIV-infected patients [56]. For instance, Cinque and colleagues reported a positive correlation between the levels of CCL2 in cerebrospinal fluid of patients with the severity of HIV-1 encephalitis [57]. In another instance, role of CCL-2 has been shown in enhancing the replication of HIV-1 in PBMCs isolated from patients [58]. These monocyte subsets (CD14++CD16- and CD14+CD16++) have been also reported in HCV infection demonstrating that CD16- monocytes may play important role in viral diseases [59,60].

2.1. Activation Status of Macrophages and HIV-1 Infection

Monocyte derived macrophages exhibits two distinct types of polarization states depending upon the presence or absence of specific microenvironment stimuli including cytokines. Interestingly, these cytokines also govern HIV-1 pathogenesis. These activation states (classically activated (M1) and alternatively activated macrophages (M2)) play an important role in mediating an effective immune response against infectious agents including HIV-1 [61–65] (Figure 1). The M1 macrophages are activated by a high amount of Th1 cytokines (IFN-γ, IL-2, IL-12), pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-18) and chemokines (CCL3, CCL4, CCL5) that enhance viral replication and block viral entry to prevent superinfection in infected macrophages [64] (Figure 1). M1 macrophages express classical pro-inflammatory cytokines such as TNF-α while M2 macrophages produce anti-inflammatory cytokines such as IL-4, TGF-β and IL-10 by a high amount [62]. During early stages of infection, the M1 macrophages are predominant which cause the tissue injury specifically in lymph nodes that is correlated with T cell apoptosis [66]. However, at later stages of viral infection, there is a shift of M1 to M2 due to the presence of IL-4 and IL-13. The M2 macrophages favor tissue repair and help to clear the opportunistic infections during HIV-1 infection. The progression of HIV-1 infection is accompanied by depletion of CD4+ T cells, resulting in frequent opportunistic infections and the imbalance of Th1 and Th2 responses leads towards the progression of AIDS [64,67].
2.2. HIV-1 Dynamics in Monocytes/Macrophages: Viral Persistence and Reservoirs

The studies on viral dynamics in monocytes demonstrate that the viral decay in monocytes is slower than that in activated CD4+ T cells. The mean half-life of viral DNA in monocytes/macrophages is longer than that in activated and resting CD4+ T cells suggesting the monocytes/macrophages as an important source of ongoing viral replication in HIV-1-infected patients on cART [68]. Findings suggest that in naive patients, the activated CD4+ T cells accounts for most of plasma viremia (99%) while the other 1% of the virus may be generated primarily from tissue macrophages [69]. However, in the presence of cART, macrophages are likely the main source of plasma viremia as active viral replication is halted in CD4+ T cells [69–71]. Furthermore, it has been reported that circulating monocytes are not a major reservoir of HIV-1 in elite suppressors [72].

2.3. Monocytes/Macrophages versus CD4+ T Cells in HIV-1 Infection

Monocyte/macrophages facilitate the transmission and establishment of HIV-1 infection to the CD4+ T cells. Macrophage-tropic HIV-1 variants have been detected during all stages of HIV-1 infection [73]. The chemokine receptor CCR5 is the principal coreceptor for macrophage-tropic HIV-1 on CD4+ T cells and monocytes/macrophages. Several macrophage-tropic variants such as HIV-1_{BAL} (lung macrophages), HIV-1_{JR-FL} (isolated from brain tissue), and HIV-1_{Ada} (from PBMCs) have been isolated.
from HIV-1 infected patients [74–76]. Several studies have demonstrated that monocytes contain HIV-1 variants that are genetically distinct from those observed in CD4+ T cells. Furthermore, the HIV isolates present in monocytes/macrophages are genetically identical or closely associated with viral variants found in the blood of suppressive cART-treated patients for longer periods of time [77,78]. Furthermore, phenotypic studies show that HIV-1 in circulating blood monocytes represents diverse viral phenotypes with multiple coreceptor and cell tropism usage during HIV-1 infection [79,80].

It is worth mentioning that opportunistic pathogens such as *Mycobacterium avium* and *Pneumocystis carinii* activate the macrophages and induce HIV production from infected macrophages in lymph nodes [81,82]. These findings suggest that macrophages can be a prominent source of viremia at later stages of HIV when lymphoid tissues are quantitatively and qualitatively impaired and opportunistic pathogens fuel HIV pathogenesis by activating and increasing the viral production from infected macrophages [40,71,81,82]. In addition, T cells also induce HIV-1 replication in myeloid cells. For example, HIV-1 replication in J22-HL-60 (promonocytic cell line) has been reported following direct contact with MOLT-4 T cells, providing the insight into the molecular mechanisms that regulate virus production from monocytes/macrophages which are latently infected with HIV-1 [83]. Moreover, macrophages selectively capture and engulf virally infected CD4+ T cells, a phenomenon that may contribute to the formation or persistence of viral reservoirs [84,85].

### 3. Modulation of Macrophage Biology by HIV-1

The life span of macrophages varies greatly and depends upon their tissue location. The tissue macrophages are long lived with a half-life of six weeks to several years. The cells of monocyte-macrophage lineage are highly resistant to viral cytopathic effects and apoptosis, and exhibit longer life spans even when they are exposed to different oxidative stress stimuli [86–88]. The macrophages of central nervous system such as microglia and perivascular macrophages produce and release toxins that induce apoptosis of neurons and astrocytes, contributing to the HIV-1-associated dementia [87,88–91].

It is worth mentioning that HIV-1 infection differentially regulates the telomerase activity in immune cells. Several studies reported that HIV-1 negatively regulates the telomerase activity in CD4+ T cells, CD8+ T cells and Jurkat T cells [92,93]. Furthermore, HIV-1 elite suppressors have longer telomeres and have higher telomerase activity [94]. Interestingly, a study has recently reported that HIV-1 infection of macrophages increases their telomerase activity. The increase in telomerase activity was specific to HIV-1 infection and correlated with p24 antigen production [95,96]. Moreover, increase in telomerase activity by either HIV-1 infection or by overexpression of human telomerase results in higher resistance of macrophages against oxidative stress and DNA damage. Collectively data suggest that HIV-1 infection of macrophages provides better protection against oxidative stress which could be an important viral strategy to make HIV-1-infected macrophages long lived and more resistant viral reservoirs (Figure 2). Furthermore, HIV-1 infection of macrophages favors the expression of macrophage colony stimulating factor (M-CSF) [97]. M-CSF is a prosurvival cytokine that down-regulates TNF-related apoptosis inducing ligand (TRAIL-R1/DR4) and upregulates the anti-apoptotic genes such as Bfl-1 and Mcl-1. Subsequently HIV-1 infected macrophages are resistant to apoptosis induced by TRAIL [97].
**Figure 2.** Macrophages fuel HIV-1 pathogenesis. HIV-1 infected macrophages secrete pro-inflammatory cytokines and chemokines that attract T cells in their vicinity, thereby transmitting virus to uninfected CD4+ T cells. Infected CD4+ T cells die soon (due to viral cytopathic effects or antiviral immune response) or return into memory CD4+ T cells as latent viral reservoirs. HIV-1 infected macrophages secrete soluble CD23 and ICAM that results in CD4+ T cell activation favoring the viral infection to CD4+ T cells. Viral gp120 increases the expression of TNF-α and TNFR2 in macrophages and T cells, resulting in CD8+ T cell apoptosis. Bystander CD4+ T cell apoptosis is triggered by FasL ligation to Fas receptor. HIV-1 infection of macrophages enhances its telomerase activity. HIV-1 expands macrophage survival by upregulating antiapoptotic genes. The P-glycoprotein transporter present on macrophages pumps out the antiretroviral drugs and limits the distribution of antiretroviral drugs to macrophages. Furthermore, macrophages spread the virus to CD4+ T cells through virological synapses. HIV-1 infected macrophages store virus into the intracellular cytoplasmic compartments providing the protection against antiviral immune response. HIV-1 infection of macrophages results in the secretion of pro-inflammatory cytokines and chemokines that ultimately accounts for the perturbation of immune trafficking.

In addition, HIV-1 infection of macrophages has been shown to modulate apoptosis and promote infection of resting CD4+ T cells. In macrophages, Nef activates a variety of signaling pathways that leads to the infection of bystander CD4+ T cells and hence expands viral reservoirs. Nef-expressing macrophages enhance resting CD4+ T cells infection through multiple cellular and soluble interactions involving macrophages and T cells [40,98]. Nef interacts with apoptosis signal regulating kinase-1...
(ASK-1) and inhibits Fas- and TNF receptor-mediated apoptosis in HIV-1-infected CD4+ T cells [40,99]. Reports suggest that the survival of infected CD4+ T cells requires intercellular contacts between macrophages and CD4+ T cells, and expression of Nef [100].

On the other hand, HIV-infected macrophages have been shown to induce apoptosis in uninfected CD4+ T and CD8+ T cells. In vitro experiments demonstrated that apoptosis inducing ligands expressed by macrophages govern apoptosis of uninfected CD4+ T cells [101–103]. The expression of TNF-α and TNFR increases during HIV-1 infection and is associated with the depletion of T cells. Following HIV-1 infection activated macrophages release TNF-α as a soluble factor or expressed as a membrane-bound form that binds to TNFR2. The binding of TNF-α to TNFR2 triggers apoptosis in CD8+ T cells [40,104,105]. In contrast to CD8+ T cells, TNFR2 is not increased on CD4+ T cells, and the apoptosis of CD4+ T cells is mediated through the interaction of Fas and FasL [40,106]. Furthermore, HIV-1 Tat upregulates the production of TRAIL in macrophages and results in the apoptosis of bystander CD4+ T cells [107]. Moreover, the binding of gp120 to CXCR4 upregulates the expression of membrane bound TNF-α and TNFR2 in macrophages and CD8+ T cells respectively (Figure 2). The binding of TNF-α and TNFR2 is associated with decreased intracellular level of Bcl-XL resulting in apoptosis of CD8+ T cells [108].

4. Macrophages Disseminate HIV-1 to CD4+ T Cells

HIV-1 infected macrophages contribute significantly to the pathogenesis of HIV infection through transmission of virus to CD4+ T cells [42] (Figure 2). It has been reported that HIV-1 infected macrophages fuse and transmit virus to CD4+ T cells through virological synapses [109–112]. In addition to virological synapses, HIV-1 infected macrophages also secrete viral containing exosomes and microvesicles that facilitate and enhance HIV-1 dissemination to uninfected CD4+ T cells [44]. The production of chemokines by HIV-1-infected monocytes/macrophages favors the recruitment and the activation of a variety of immune cells (Figure 2). In vitro, HIV infection of macrophages leads to the production of several chemokines such as CCL-2, CCL-3, CCL-4 and CCL-5 [113–115] which in turn favor the recruitment of immune cells including monocytes, macrophages, dendritic cells and T cells. The HIV-1 Nef protein plays a critical role for this function. The adenovirus-mediated expression of Nef in macrophages induces chemokine production that results in chemotaxis and activation of CD4+ T cells for productive HIV-1 infection [116–118]. In addition, HIV-1 Nef intersects the macrophage CD40L signaling pathway and promotes the resting CD4+ T cell infection by inducing soluble CD23 and soluble ICAM [119].

5. Macrophage Infection under cART

The activity of different antiretroviral drugs has been investigated in macrophages chronically infected with HIV-1 [120,121]. Protease inhibitors (PIs) have been shown to be a powerful therapeutic tool to fight HIV infection [122,123]. The combination of PIs along with reverse transcriptase inhibitors has the ability to target the viral replication at early and late stages of HIV infection. The activity of PIs such as saquinavir and ritonavir on HIV-1 infection in monocytes/macrophages was found to be several folds lower than in T cells [120]. Furthermore, the intracellular concentrations of active metabolites of nucleoside analogs were significantly lower (5 to 140 fold) in macrophages than in lymphocytes. The
high expression of P-glycoprotein transporter in macrophages has been reported to limit the availability and absorption of these drugs [124–126]. This remarkable feature renders the macrophages resistant to certain antiretroviral drugs and ultimately promotes the emergence of viral escape mutants [127,128]. Furthermore, pharmacological inhibition of P-glycoprotein transport enhances absorption and distribution of HIV-1 protease inhibitors to different organs [129,130]. The relatively lower antiviral activity of anti-HIV drugs in macrophages allows continued HIV-1 replication, which may result in the formation of HIV-1 reservoirs and emergence of resistant virus.

In situ hybridization studies on simian immunodeficiency virus HIV type 1 chimera (SHIV) showed that the tissue macrophages in lymph nodes contain high plasma virus in the absence of CD4+ T cells [131]. Quantitative analysis reveals that most of virus producing cells (95%) in these tissues are macrophages and 2% are T lymphocytes. In addition, the administration of potent HIV reverse transcriptase inhibitors blocked the virus production during early infection in T cells but not in macrophages [131]. During macrophage infection, the presence of an individual mutation in HIV integrase is sufficient to produce virus resistant to raltegravir [132]. A recent study by Micci and co-workers demonstrated that the macrophages act as a prominent source of virus in the rhesus macaques that were experimentally depleted of CD4+ T cells followed by SIV infection [133]. Altogether, these different lines of evidence demonstrate that macrophages provide a favorable environment for HIV persistence [133,134].

6. Cellular Restrictions Factors and HIV Replication in Macrophages

The importance of macrophages in HIV-1 pathogenesis is further underlined with the discoveries of the presence of anti-HIV-1 cellular restriction factors. Some restriction factors were found to be macrophage-specific and some play role in several cell types. SAMHD1 (sterile alpha motif domain- and HD domain-containing protein 1) is a cellular restriction factor that restricts the replication of HIV-1 and Vpx deficient HIV-2 [135,136]. Noteworthy, SAMHD1 is not specific for macrophages and was initially reported as restriction factor in dendritic cells and apparently also plays a role in CD4+ T cells. SAMHD1 has dNTPase activity that significantly reduces the dNTPs pools, thereby limiting the reverse transcriptase (RT) activity of HIV. Vpx protein of HIV-2 has been shown to promote proteasome dependent degradation of SAMHD1 [135]. Despite of absence of Vpx in HIV-1 genome, virus successfully replicates in the macrophages. Recently, Kyei and colleagues reported the direct involvement of cyclin L2 in triggering the proteasomal degradation of SAMHD1 [137]. In addition to SAMHD1, p21 (also called CDKN1A) has been shown to restrict the replication of HIV-1 in MDMs by governing the expression of ribonucleotide reductase subunit R2 [138]. This resulted in the decreased intracellular dNTPs pools limiting the RT activity of HIV-1 [138]. Several other HIV-1 restriction factors have been described including APOBEC3A, APOBEC3G [139–143], tetherin [144,145], TRIM5-alpha [146] and MX2 [147] suggesting the significant importance of macrophages in HIV-1 pathogenesis.

7. Post-Integration Reactivation of HIV from Macrophages

Post integrated HIV-1 DNA has been well characterized in macrophages at least in vitro and to lesser extent in vivo [148]. Barr et al. sequenced and analyzed 754 unique integration sites in macrophages infected with HIV-1 in vitro. They found the preferential integration of HIV-1 in active transcriptional units [149]. HIV-1 was found to be integrated in Toll-like receptor and CAP-binding protein complex
interacting homologue genes [150]. The viral replication in monocytes isolated from HIV-1 patients under cART has been reported [151,152]. However, whether HIV-1 was in unintegrated or integrated form was not characterized [152].

Figure 3. Therapeutic approaches could favor the clearance of HIV-1 from macrophage reservoirs. Macrophages harbor integrated as well as unintegrated proviral DNA. Antiretroviral therapy interferes with several steps of HIV-1 life cycle including entry, reverse transcription, proviral DNA integration, polyprotein processing and release of viral progeny. HIV-1 infection also results in the establishment of latency in less studied reservoirs (macrophages). Macrophages harboring latent HIV-1 [157,158] can be activated by variety of approaches including chemokines, cytokines and HDACi. In addition several apoptotic reagents have been also employed which can specifically induce apoptosis in infected macrophages in vitro [44].

Several latently infected cell lines have been routinely used to study the HIV latency, such as U1 cells. Proinflammatory chemokines like TNF alpha and HDAC inhibitors (HDACi) have been found to be effective in reactivating HIV-1 in these model latent cell lines in vitro (Figure 3). For instance, HDACi givinostat, belinostat and panobinostat have been shown to decrease the expression of HIV-1 coreceptor CCR5 and to increase viral growth in U1 cells [153]. In another instance the bromodomain inhibitor JQ1 has been shown to reactivate HIV-1 in U1 cells [154]. However, the impact of biological or pharmacological HIV-1 inducers such as HDACi could be difficult to assess in latently infected
macrophages. The presence of multidrug pumps in macrophage and inability to reach the tissue specific macrophages in sufficient concentration could contribute to the ineffectiveness of HIV-1 inducers in reactivating HIV-1 in macrophages in vivo [155,156]. The study of drugs reactivating HIV from latently infected monocytes/macrophages such as HDACi and bromodomain inhibitors and apoptosis inducing agents [44] need further investigation especially in vivo in order to potentially clear HIV-1 from the cellular reservoir in HIV-infected patients (Figure 3).

8. Conclusions

There are several reasons that explain why macrophages play an important role in the pathogenesis of HIV-1. From HIV standpoint, macrophages provide an ideal environment for the formation of viral reservoirs since they live long, are widely distributed throughout the body and are relatively resistant to HIV-induced apoptosis. Moreover, HIV-1 infection enhances the survival of macrophages by upregulating antiapoptotic genes. HIV-1 infection of macrophages activates host transcription factors such as NF-kB and prevents the macrophages from TNF-induced apoptosis. Furthermore, virally infected macrophages secrete CC-chemokines that attract the T lymphocytes in their vicinity leading to their productive viral infection. In addition, activated macrophages could favor the depletion of both uninfected CD4+ T cells and CD8+ T cells leading to immune deficiency. Altogether, macrophages play a critical role in HIV pathogenesis by expanding the viral reservoir that ultimately fuels disease progression. HIV-infected monocytes/macrophages are less sensitive to cART as compared to infected CD4+ T cells. Therefore the development of new therapeutic approaches to clear HIV from monocyte/macrophage reservoirs is under way although total clearance of HIV from macrophage reservoirs is still an uncertain goal that needs to be reached in the future to definitively cure HIV-infected patients.

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Author Contributions

WA and AK were responsible for writing the manuscript. WA and AK created the figures. MT and MI were responsible in organizing the contents and also assisted in revising the manuscript. GH was involved in critical reading of the manuscript. All the authors read and approved the final manuscript.

List of Abbreviations

HIV-1: human immunodeficiency virus type-1, TNF-α: tumor necrosis factor alpha, TGF-β: transforming growth factor beta, IL: interleukin, IFN-γ: interferon gamma, cART: combination anti-retroviral therapy, AZT: azidothymidine, TRAIL: TNF-related apoptosis-inducing ligand, ASK-1: apoptosis signal
regulating kinase-1, ICAM: intercellular adhesion molecule, SAMHD1: sterile alpha motif domain- and HD domain-containing protein 1, HDAC: histone deacetylase, HDACi: HDAC inhibitor.

Conflicts of Interest

The authors declare no conflict of interest.

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