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Role of myeloid cells in HIV-1- host interplay

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Abstract

The AIDS Research Field has embarked on a bold mission to cure HIV-1-infected individuals of the virus. To do so, scientists are attempting to identify the reservoirs that support viral persistence in patients on therapy, to understand how viral persistence is regulated and to come up with strategies that interrupt viral persistence and that eliminate the viral reservoirs. Most of the attention regarding the cure of HIV-1 has infection has focused on the CD4+ T-cell reservoir. Investigators are developing tools to probe the CD4+ T-cell reservoirs as well as *in vitro* systems that provide clues on how to perturb them. By comparison, the myeloid cell, and in particular, the macrophage has received far less attention. As a consequence, there is very little understanding as to the role played by myeloid cells in viral persistence in HIV-1 infected individuals on suppressive therapy. As such, should myeloid cells constitute a viral reservoir, unique strategies may be required for their elimination. This article will overview research that is examining the role of macrophage in virus-host interplay and will discuss features of this interplay that could impact efforts to eliminate myeloid cell reservoirs.

Keywords

HIV-1; reservoirs; macrophage; eradication

Macrophage reservoirs of primate lentiviruses

The earliest report of HIV-1 infection of tissue macrophages was detailed by Gartner and colleagues (Gartner et.al.1986). That study provided evidence that macrophage from brain and lung tissue harbored the virus and that macrophages from blood and bone marrow of healthy donors could replicate the virus *in vitro*. That study also provided the earliest indication that HIV-1 exhibited differential tropism for macrophages and CD4+ T-cells. Since that pioneering study, progress into defining macrophage reservoirs in HIV-1-infected individuals has been slow. Furthermore, despite numerous demonstrations that macrophages can be infected with primate lentiviruses in vitro, there has been little attention or interest in establishing whether macrophage reservoirs are important for the maintenance of infection particularly in the face of antiretroviral suppression. The issue of macrophage reservoirs in the CNS has been comprehensively reviewed elsewhere, (for example, see Churchill and Nath 2013) and will not be covered here. Lack of attention on macrophage reservoirs no doubt stems from the difficulty in accessing tissue macrophage for detailed study. Jambo

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and colleagues recently demonstrated that small alveolar macrophages from viremic, HIV-1-infected individuals harbor HIV-1 at surprisingly high frequencies relative to CD4+ T-cells (Jambo et. al. 2014). Infected cells were identified by fluorescence in situ hybridization (FISH) for viral transcripts. This demonstrates that infected macrophages were transcriptionally active but also indicates that if HIV-1 can establish latent infection in macrophages, FISH might underestimate the frequency of infected macrophages in the lung. If indeed, the alveolar macrophages are a reservoir, this more accessible source of tissue macrophages could facilitate studies to examine the role played by macrophages in viral persistence.

Are macrophages a viral reservoir in individuals on suppressive ART? There has already been some attention paid as to whether antiretroviral agents are equally effective in macrophages as in CD4 T-cells (reviewed in Gavegnano and Schinazi, 2009). Whether macrophages can provide a sanctuary from antiretroviral suppression is an important but understudied issue. Fletcher et al. (2014) demonstrated that the intracellular concentration of some antiretroviral agents in cells from lymphoid tissue of individuals on suppressive ART are lower than those in peripheral CD4 T-cells (Fletcher et al. 2014). It is not yet known whether antiretrovirals achieve similar concentrations in tissue macrophages in lymphoid tissue as in peripheral CD4 T-cells. In addition to this, the debate continues on whether macrophages from individuals on suppressive ART harbor HIV-1. Yukl and colleagues examined the distribution of viral DNA in blood, ileum and rectum from virologically suppressed individuals (Yukl et al. 2013). HIV-1 DNA, and to a lesser extent RNA was detected in non-CD4+ T-cells from the gut but was barely detectable in non-CD4+ T-cells from blood. Yukl and colleagues went on to determine the identity of the non-CD4+ T-cells harboring viral DNA using cell sorting and identified them as macrophages (Yukl et al. 2014). While these studies provide intriguing evidence that tissue macrophages from individuals on suppressive ART harbor viral DNA, they do not provide definitive evidence that macrophages are a viral reservoir. Macrophages recognize and phagocytose cells undergoing apoptosis but not normal cells. Apoptotic cell surface phosphatidylserine is recognized by CD14 on the macrophage, resulting in engulfment. In uncontrolled replication, one could envision preferential engulfment of apoptotic, HIV-1 infected cells by macrophages and as a consequence, the viral DNA detected in sorted myeloid cells could have been the result of ingestion of an infected CD4+ T-cell (see caveats below). Therefore, additional analysis such as phylogenetic comparison of viruses in macrophages and CD4+ T-cell populations, or demonstration that envelopes from macrophages exhibit low CD4 dependence (Joseph et al. 2014), would provide additional evidence that sequences detected in macrophages were generated by infection of the macrophage (see caveats below). The studies by Yukl and colleagues (Yukl et al. 2014) suggest that viral RNA is far less abundant in myeloid cells from virologically suppressed individuals. Therefore, it would be appropriate to determine whether tissue macrophages isolated from individuals on suppressive ART can produce infectious virus when cultured *ex vivo*. If virus can be recovered, it would suggest that these macrophages harbor latent virus. These questions might be more tractable using alveolar macrophages. There are important caveats to consider when assigning the origin of proviral DNA in macrophage to phagocytosed, HIV-1-infected CD4 T-cells. Firstly, phagocytosed cells would have to exhibit “eat me”

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signals (phosphatidylserine) that trigger engulfment by macrophages. In uncontrolled viral replication, HIV-1 infected cells undergoing cytopathic infection are likely to exhibit engulfment signals. However, in individuals on suppressive ART, and where infected cells are in a minimally productive or latent state of infection, there is unlikely to be any cell surface feature distinguishing the infected cell from an uninfected cell. As such, one would not expect selective engulfment of infected cells over normal cells. Furthermore, in cells undergoing apoptosis, cellular DNA is rapidly subject to nuclease digestion prior to and after engulfment of the apoptotic cell by macrophages (reviewed in Samejima and Earnshaw, 2005). Within hours, nucleases cleave DNA within the apoptotic cell to fragments of 0.5–2 kilobases. This occurs even prior to engulfment. A separate set of nucleases further cleaves DNA of the apoptotic cell within phagolysosomes. It is therefore difficult to envision a scenario where proviral DNA within an engulfed CD4 T-cell would remain intact long enough to be registered by PCR. However, use of PCR with primers that generate HIV-1 amplicons larger than several kilobases would more specifically identify proviruses within macrophages as opposed to fragmented DNA remnants of engulfed, apoptotic CD4 T-cells.

Are myeloid cells differentially restricted to infection by HIV-1 and HIV-2/SIV?

Macrophages and T-cells are not completely passive to infection by primate lentiviruses. T-cells and macrophage-encode proteins, also known as cellular restrictions that antagonize viral replication. Cellular restrictions with activity for HIV-1 in human cells include the Apobec 3 family of cytidine deaminases, tetherin/Bst2 and SAMHD1 (for recent review, see Ayinde et al. 2012). In order to replicate within their primate hosts, primate lentiviruses have evolved mechanisms to neutralize the antiviral activity of these cellular restrictions. The viral accessory proteins play a central role in defense strategies used by primate lentiviruses to oppose cellular restrictions. The Apobec 3 proteins are neutralized by the vif protein, tetherin/Bst2 is neutralized by vpu (or by nef in viruses lacking vpu) and SAMHD1 is negated by vpx. Research on vpx and its impact on infection of macrophages has generated a paradox that, taken at face value, might indicate that macrophages are irrelevant in the interplay between HIV-1 and its host. Early studies demonstrated that vpx of HIV-2/SIV was necessary for infection of monocyte-derived macrophages (MDM) but dispensable for infection of CD4+ T-cells (Fletcher et al. 1996). Subsequently, Sharova and colleagues provided evidence that vpx supports viral infection of macrophages by counteracting a dominant cellular restriction (Sharova et al. 2008). The cellular restriction that is targeted by vpx was recently identified as SAMHD1 (Laguette et al. 2011; Hrecka et al. 2011). SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase (dNTPase) that reduces cellular dNTP levels (reviewed in Wu, 2013). In the presence of SAMHD1, dNTP levels are too low to support lentiviral reverse transcription. Vpx proteins that are packaged in the virion, promote ubiquitylation and proteasomal destruction of SAMHD1 and as such, maintains conditions for the generation of the viral cDNA. Prior to the identification of SAMHD1 as the restriction, Sharova and colleagues demonstrated that the restriction was active against HIV-1 but that HIV-1 vpr, which is the ortholog to vpx of HIV-2/SIV, was unable to neutralize the restriction (Sharova et al. 2008). The fact that HIV-1 is antagonized

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in macrophages but that HIV-1 has no apparent ability to neutralize the restriction (Table 1) creates a paradox. If macrophages are indeed important for the replication and persistence of HIV-1 in its host, one would expect that HIV-1 has evolved a mechanism to neutralize the restriction. There are several conclusions that could be drawn from this; macrophages are not important targets for HIV-1 replication and as such, the virus does not have to defend itself against macrophage restrictions; HIV-1 has evolved a mechanism to reverse transcribe viral cDNA in the low dNTP environment created by SAMHD1. If the latter, it would indicate that reverse transcriptase of HIV-1 is functional in a low dNTP environment while SIV reverse transcriptase is not, although there is no direct experimental evidence to support differential sensitivity of HIV-1 and SIV reverse transcriptases to low dNTP conditions. While the mechanism by which HIV-1 escapes full antagonism by SAMHD1 is unknown, the HIV-1-SAMHD1 axis remains an unexplored therapeutic target. Rendering HIV-1 fully sensitive to SAMHD1 restriction would be predicted to render macrophages non-permissive to HIV-1 infection. The bigger question is why HIV-1 has not adopted similar strategy to HIV-1/SIV to evade SAMHD1. It is possible that HIV-1 has another mechanism to survive in the hostile conditions created by SAMHD1. However, HIV-1 is clearly subject to the effects of SAMHD1 during infection of macrophages (Sharova et al., 2008) yet chooses not to completely defend itself. Therefore, it is possible that some degree of antagonism by SAMHD1 might be advantageous to HIV-1. For example, we have evidence that conferring upon HIV-1 the ability to completely neutralize SAMHD1 (by packaging vpx within HIV-1 virions) dramatically increases efficiency of macrophage infection. It is possible that such an efficient infection may be less favorable to the establishment of a persistent and non-cytopathic infection and that HIV-1 was evolutionarily pressed not to completely oppose SAMHD1 in order to better persist within the host. Of course, a prediction of this is that HIV-1 is inherently more cytopathic than HIV-2/SIV.

Obstacles to elimination of myeloid cell reservoirs

Rapid turnover of productively infected CD4+ T-cells is a central feature of HIV-1 infection and is a consequence of cytopathic virus replication as well as the elimination of infected cells by the cellular immune response (reviewed in Perelson 2002). Strategies being explored for elimination of viral reservoirs from infected individuals on suppressive ART center on the premise that infected cells, if undergoing productive infection, should succumb to these clearance forces. This has given rise to so-called “purging” or “kick and kill” therapeutic approaches that attempt to stimulate viral activity to the point that cytopathicity and cellular immunity eliminate the infected cell. CD4+ T-cells from individuals on suppressive ART harbor the virus in a latent or minimally active state (reviewed in Ruelas and Greene, 2013). Since viral latency is primarily considered to be regulated by histone architecture, drugs that relax chromatin are being explored for their ability to reactivate the virus from latency (reviewed in Manson McManamy et al. 2014). Chromatin modifiers such as vorinostat can reactivate viral latency in *in vitro* models and there is some evidence for weak activation of the virus *in vivo* (Archin et al. 2012). Most of the attention has been on whether purging protocols can impact the CD4+ T-cell reservoir. However, it is not clear that myeloid cell reservoirs would be amenable to elimination by the approaches that are

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being explored to eliminate CD4+ T-cell reservoirs and prior research suggests they might not.

Cytopathicity is a well-recognized consequence of viral replication in CD4+ T-cells and is manifest as syncytium formation and single cell killing. Whether syncytia between infected and uninfected CD4 T-cells occurs in infected individuals is not known. Furthermore, the relative contribution of direct killing of infected cells by syncytial formation or single-cell killing versus bystander cell death to CD4 turnover in HIV-1-infected individuals, is not well understood. However, there is no obvious cytopathicity during efficient HIV-1 replication in MDM (Swingler et al. 2007). Several labs including my own have explored the basis for the apparent resistance of MDM to HIV-1 cytopathicity. For example, we have demonstrated that HIV-1 replication in macrophages results in induction of the survival cytokine, monocyte colony stimulating factor (MCSF), (Swingler et al. 2007). However, cytopathic infection is observed upon inhibition of MCSF signaling. We have demonstrated that Gleevec, a low affinity inhibitor of MCSF receptor (c-fms) signaling, can induce cytopathic infection of macrophages (Swingler et al. 2007). Intriguingly, recent studies from the Merad laboratory suggest that tissue resident macrophages repopulate locally (rather than from circulating monocytes) in an MCSF and Granulocyte macrophage (GM)-CSF-dependent manner (Hashimoto et al. 2013). Recent studies further suggest that tissue macrophages, including microglia and Kupffer cells, have a bone-marrow independent origin (Perdiguero and Geissmann, 2014). It is possible that activation of HIV-1 activity in macrophages will not be sufficient to drive clearance via cytopathicity but that use of agents such as MCSF may sensitize infected macrophage to viral cytopathicity. It may also be possible to harness the phagocytic property of macrophages to promote their clearance. Cytotoxic drugs such as clodronate, have been encapsulated in liposomes and delivered to macrophages via phagocytosis. This strategy has been explored as a way to deplete macrophages in experimental models of disease (reviewed in Meyer et al. 2012). Although blood monocytes are also reduced by this approach, local administration of liposomes would avoid this. For example, liposomes with cytotoxic cargoes could be administered to the lung by aerosol thereby eliminating infected alveolar macrophages.

The premise that cell mediated immunity will clear infected cells after virus reactivation might also be problematic when dealing with the macrophage reservoir. Cytotoxic T cells can impact viremia and drive emergence of resistant viruses with mutations in CTL epitopes (reviewed in Goulder and Watkins, 2008). However, the extent to which infected CD4+ T-cells, once reactivated from latency, are eliminated by the cellular immune response, is not known. Using an *in vitro* model of HIV-1 latency, Shan and colleagues examined the ability of HIV-1-specific cytolytic T cells to kill CD4+ T-cells following reactivation of latent infection with the histone deacetylase inhibitor SAHA (Shan et al. 2012). Following latency reactivation, CD4+ T-cells neither succumbed to viral cytopathicity or to killing by autologous cytolytic T-cells. However, antigen-specific stimulation of cytolytic T-cells from infected individuals rendered those cells effective in killing latently infected cells after their reactivation (Shan et al. 2012). One caveat to this study is that target cells were transfected with Bcl-2 to allow their long-term culture and this might also influence susceptibility of the cells to killing by cytolytic T-cells. Somewhat paradoxically, in virus outgrowth assays, *ex*

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vivo rescue of latent HIV-1 from quiescent CD4+ T-cells of individuals on suppressive ART requires removal of CD8 cells prior to virus rescue. Therefore, un-stimulated CD8 cells appear capable of suppressing virus outgrowth in this setting.

Whether cytolytic cells are capable of controlling virus replication in macrophages, either during productive infection or following reactivation from latency, is not known. In collaboration with the laboratory of David Watkins, we have been assessing the impact of SIV-specific CD8+ T cells on SIV replication in macrophages. The advantage to addressing this issue in the SIV/macaque model is the ability to compare antiviral activity of CD8+ T cells in MHC class I- matched and mismatched targets. Tetramer sorted SIV-specific CD8+ T cells were able to suppress viral replication in SIV-infected MHC class I-matched CD4+ T cells but not in MHC class I-mismatched CD4+ T cells (Vojnov et al. 2012). In contrast, *ex vivo* tetramer sorted CD8 T cells failed to impact viral replication in MHC class I-matched, SIV-infected macrophages that were derived from monocytes and differentiated *in vitro* (Vojnov et al. 2012). While HIV-1 and SIV-specific CD8+ T cell lines and clones have been demonstrated to kill infected macrophages, the biological activities of these cell lines and clones may not be representative of the activities of fresh virus-specific CD8 T cells nor of primary macrophage targets. Of the biological activities described for the viral nef accessory protein, down-regulation of cell surface expression of MHC class I molecules is the best studied (reviewed in Wonderlich et al. 2013). Nef disrupts trafficking of MHC class I molecules to the cell surface thereby limiting antigen presentation and providing the infected cell some measure of escape from CD8+ T cell surveillance. MHC class I densities on macrophage are lower than on T cells and further nef- mediated reduction in class I expression on the surface of infected macrophage may contribute to the lack of sensitivity of infected macrophage to CD8+ T cell killing. These issues are currently under investigation.

In vitro systems to study viral persistence in macrophage

Research on viral latency and persistence has evolved away from using cell line-based models to primary cell models (reviewed in Spina et al. 2013). While primary cell models of viral latency are technically more challenging, they likely offer greater biologically relevant insight into how viral latency is regulated. For establishment of a latent infection, cells must be in a physiologic state that is permissive to infection yet not succumb to viral cytopathicity. Some investigators have used cytokines to render primary CD4+ T cells permissive to infection without inducing their full activation since infection of fully activated cells invariably leads to cytopathicity (Saleh et al. 2011). The result is a cell that supports virus infection and entry into latency. Other groups have established primary cell models of latency in memory CD4+ T cells (Bosque and Planelles 2009). Some of these primary cell models are also being used in small molecule screens for agents that can reactivate viral latency (reviewed in Margolis and Hazuda, 2013). Studies of myeloid cell latency have predominantly employed myeloid cell lines (reviewed in Redel et al 2010) and have demonstrated that, similar to CD4+ T cell latency, latent HIV-1 can be reactivated from myeloid cell lines using agents that activate the NF-kB pathway. Data in primary cells is lacking as is data that myeloid-lineage cells harbor latent virus in HIV-1-infected individuals. However, macrophage exhibit characteristics that would appear ideally suited to the establishment of latency. Macrophage are resistant to viral cytopathicity (Swingler et al.

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2007) therefore, once infected, macrophage are more likely to progress to a state of latent or persistent infection than to be eliminated by cytopathicity. Macrophage may also be resistant to cell-mediated killing (Vojnov et al., 2012) that may also contribute to the establishment of latency. Studies on viral CD4+ T cell reservoirs in HIV-1-infected individuals on ART indicate that homeostatic proliferation might provide a mechanism to maintain the viral CD4+ T cell reservoir (Chomont et al 2009). Evidence for homeostatic maintenance of viral reservoirs is provided indirectly by the clonal nature of viruses that populate plasma in individuals with low level viremia on suppressive ART (Bailey et al 2006). This is most likely explained by recrudescence of virus from identical proviruses that were generated through numerous rounds of mitosis. It has been assumed that tissue macrophage do not divide and thus, maintenance of latent virus might be due to the intrinsic stability of the macrophage. However, mouse studies on mechanisms of macrophage homeostasis suggest that tissue macrophage self-renew locally through proliferation and with minimal contribution from circulating monocytes (Hashimoto et al., 2013). Current models suggest that perivascular and resident tissue macrophage (including microglia, peritoneal macrophage, alveolar macrophage, kupffer cells, bone marrow macrophage) derive from circulating monocyte precursors (reviewed in K. Takahashi, 2001). If relevant to humans, the studies by Hashimoto and colleagues (2013) raise the possibility that HIV-1 persistence in tissue resident macrophage is also maintained by proliferation and duplication of proviruses at mitosis. Studies from Yukl et al (2013, 2014) have demonstrated the presence of viral DNA in macrophage from rectal tissue of HIV-1-infected individuals on ART. While levels of proviral DNA in some individuals approached those in memory CD4+ T cell subsets, cell-associated viral RNA levels were low to undetectable. Therefore, viral genomes in tissue macrophage were either defective (harboring mutations that, for example, cripple viral transcription) or in a latent state. Opportunistic pathogens, including Mycobacterium Avium and Pneumocystis Carinii, can greatly increase HIV-1 expression in tissue macrophage in co-infected lymphoid tissue (Orenstein et al., 1997). This suggests that HIV-1 can be induced in tissue macrophage, at least in viremic individuals. Macrophage phenotype, and in particular phenotypes manifest in inflammatory conditions, are also likely to impact the interplay between tissue macrophage and the virus. Pro-inflammatory cytokines such as interferon-γ (IFN-γ) promote an M1 phenotype that is necessary for the killing of intracellular pathogens and tumor cells while the M2 phenotype, that is created by, for example stimulation via toll-like receptor, is important for macrophage participation in activities such as tissue remodelling and clearance of injured cells. Many studies have highlighted the impact of macrophage phenotype on the outcome of HIV-1 infection (reviewed in Alfano et al., 2013). How macrophage polarization phenotype impacts establishment of viral latency in macrophage, or reactivation from latency, warrants further study.

We have been establishing primary macrophage models in which to study mechanisms of viral persistence and latency *in vitro* using macrophages that are derived from elutriated monocytes after culture in MCSF (Heinzinger et al 1995). Macrophages derived in this way are highly permissive to HIV-1 infection. Highly productive replication continues 2–3 months post-infection followed by non-productive/latent state that can extend for as long as cells can be maintained viable (up to 6 months. During this non-productive phase, agents

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that activate virus production can be evaluated. Preliminary studies indicate that HIV-1 can be maintained in a minimally productive or latent state for many months and that virus production can be reactivated by agents that stimulate the NF-kB pathway while reactivation can be blocked by agents that antagonize NF-kB signalling. The physiologic basis for the non-productive phase in macrophages is unclear but studies from Liou et al (2002) indicate that Cyclin T1, a component of the TAK/P-TEFb kinase complex upon which tat-driven transcription is essential, becomes rate-limiting in late differentiated macrophages. Stimulation of NF-kB in macrophages via LPS restored cyclin T1 expression and HIV-1 transcription. As such, reactivation from latent/non-productive infection or prevention of viral reactivation from latency can be obtained with agents that activate or block Nf-kB signaling and cyclin T1 expression, respectively.

Conclusion

While definitive evidence for a reservoir of latently-infected macrophages in HIV-1-infected individuals on suppressive ART is work in progress, there is good data to indicate that macrophages harbor HIV-1 in such individuals and as such, a potential obstacle to eradication of HIV-1. Primary cell models that reconstitute viral latency will provide insight into whether viral persistence in macrophages can be interrupted by the same strategies being pursued to interrupt latent infection of CD4+ T cells. Furthermore, more attention needed to be given to the dynamics of macrophage reservoirs under long term ART as well as the impact of therapies that attempt to purge CD4+ T cell reservoirs. Analysis of macrophages in the lung may be provide an important window into the dynamics of macrophage reservoirs under different treatment regimens and, importantly, whether viruses in tissue macrophages from individuals on suppressive ART contribute to viral persistence. This could be approached through phylogenetic comparisons of viral sequences in tissue macrophages from individuals on suppressive ART with those in recrudescing virus in plasma following treatment interruption. Such a demonstration would provide the catalyst for greater research emphasis on approaches to eliminate the macrophage reservoir.

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Table 1

Differential restriction of primate lentiviruses by SAMHD1

SAMHD1 depletes cellular dNTP pools that are required for efficient reverse transcription of viral cDNA. Vpx of HIV-2/SIV but not Vpr of HIV-1, maintains cellular dNTP levels promoting proteasomal degradation of SAMHD1. Despite inability to antagonize SAMHD1, HIV-1 is infectious for macrophage. HIV-1 is antagonized by SAMHD1 since vpx, provided *in trans*, increases infectivity of HIV-1 for macrophage (Sharova et al. 2008). In contrast, macrophage are refractory to infection by vpx-deleted SIV and infection can be fully restored by introduction of vpx *in trans*. Since vpr of HIV-1 does not restore infectivity of vpx-deleted SIV, vpr lacks the ability to counteract SAMHD1. As such, HIV-1 retains infectivity for macrophage in the face of SAMHD1- mediated dNTP depletion.

Virus	SAMHD1 antagonism	Infectivity for macrophage
HIV-1_{wt} wt	No	Yes
HIV-1_{wt} + Vpx (*in trans*)	Yes	Yes (augmented)
SIV wt	Yes	Yes
SIV $_{Vpx}$	No	No
SIV $_{Vpx}$ + Vpx (*in trans*)	Yes	Yes