Lentiviral infection of proliferating brain macrophages in HIV and simian immunodeficiency virus encephalitis despite sterile alpha motif and histidine-aspartate domain-containing protein 1 expression

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Objective: HIV-1 infection of the brain and related cognitive impairment remain prevalent in HIV-1-infected individuals despite combination antiretroviral therapy. Sterile alpha motif and histidine-aspartate domain-containing protein 1 (SAMHD1) is a newly identified host restriction factor that blocks the replication of HIV-1 and other retroviruses in myeloid cells. Cell cycle-regulated phosphorylation at residue Thr592 and viral protein X (Vpx)-mediated degradation of SAMHD1 have been shown to bypass SAMHD1 restriction in vitro. Herein, we investigated expression and phosphorylation of SAMHD1 in vivo in relation to macrophage infection and proliferation during the neuropathogenesis of HIV-1 and simian immunodeficiency virus (SIV) encephalitis.

Methods: Using brain and other tissues from uninfected and SIV-infected macaques with or without encephalitis, we performed immunohistochemistry, multilabel fluorescence microscopy and western blot to examine the expression, localization and phosphorylation of SAMHD1.

Results: The number of SAMHD1⁺ nuclei increased in encephalitic brains despite the presence of Vpx. Many of these cells were perivascular macrophages, although subsets of SAMHD1⁺ microglia and endothelial cells were also observed. The SAMHD1⁺ macrophages were shown to be both infected and proliferating. Moreover, the presence of cycling SAMHD1⁺ brain macrophages was confirmed in the tissue of HIV-1-infected patients with encephalitis. Finally, western blot analysis of brain-protein extracts from SIV-infected macaques showed that SAMHD1 protein exists in the brain mainly as an inactive Thr592-phosphorylated form.

Conclusion: The ability of SAMHD1 to act as a restriction factor for SIV/HIV in the brain is likely bypassed in proliferating brain macrophages through the phosphorylation-mediated inactivation, not Vpx-mediated degradation of SAMHD1.

Keywords: encephalitis, HIV, Ki-67, macrophage, proliferating cell nuclear antigen, sterile alpha motif and histidine-aspartate domain-containing protein 1, viral protein X

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Introduction

Sterile alpha motif and histidine-aspartate domain-containing protein 1 (SAMHD1) is a multifunctional enzyme with deoxyribonucleoside triphosphatase triphosphohydrolase (dNTPase) and 3'-5'-exoribonuclease (RNase) activities. SAMHD1 was first identified as a gene that is responsible for Aicardi–Goutières syndrome (AGS) [1]. Its loss-of-function point mutation causes an autoimmune-like encephalopathy that mimics congenital viral brain infection [2]. It was recently shown that SAMHD1 is recruited to sites of DNA damage in DNA repair while also regulating cell proliferation and survival [3,4].

In 2011, human SAMHD1 was identified as a novel myeloid-specific restriction factor of HIV-1 that can be counteracted by viral protein X (Vpx), an accessory protein encoded by HIV-2 and related simian immunodeficiency virus (SIV) [5,6]. Shortly after its identification as an antiviral restriction factor, SAMHD1 was found to be the only known mammalian dNTPase [7,8]. Although the mechanism of action of SAMHD1 is not fully understood, current understanding is that by converting the intracellular deoxyribonucleoside triphosphates (dNTPs) to deoxyribonucleosides via its triphosphohydrolase activity, SAMHD1 depletes the intracellular pool of dNTPs to a level below that which is required for reverse transcription, thereby protecting target cells from HIV infection [9,10]. Moreover, a further search for the antiviral mechanism revealed that SAMHD1 is an RNase that binds and degrades single-stranded RNA and DNA. The RNase rather than dNTPase activity of SAMHD1 has been suggested to be responsible for its HIV-1 restrictive function by directly degrading viral genomic RNA [11,12], which remains controversial.

SAMHD1 is expressed on a variety of cell types, including HIV/SIV targets, monocyte/macrophages and CD4+ T lymphocytes [13]. It is not too difficult to speculate that virus can negatively regulate expression and/or antiviral activity of potent SAMHD1 in macrophages and CD4+ T cells as HIV and SIV still infect their SAMHD1-expressing targets in vivo.

There have been several theories as to why SAMHD1 is ineffective at restricting HIV/SIV infection in vivo. Vpx of HIV-2 and SIVmac has been shown, in vitro, to tag SAMHD1 for proteosomal degradation through its interactions with DDB1 and CUL4-associated factor 1 [5,6,14]. Other in-vitro studies [15–19] have demonstrated that SAMHD1 is phosphorylated by cyclin-dependent kinases 1 or 2 (CDK1/2) on residue threonine 592 (Thr592) in cycling cells and that this phosphorylation abrogates its HIV-1 restriction activity; whether it is its dNTPase or RNase activity that is negatively affected by the phosphorylation of SAMHD1 is still a matter of debate. However, there has been no in-vivo evidence that HIV and SIV use the above-mentioned mechanisms to counteract or bypass SAMHD1 and establish viral infection in humans and monkeys, respectively.

Previously, we demonstrated that proliferating brain perivascular macrophages (PVMs) prevail in SIV and HIV encephalitis (SIVE and HIVE) and are infected with virus [20]. We speculated that this population of PVMs is capable of being infected because of inactivation or degradation of SAMHD1. In the current study, using our rhesus macaque model of HIV infection and neuroAIDS, we set out to examine expression of the SAMHD1 and Vpx proteins in SIV-infected rhesus macaques in relation to SIV infection in vivo. We also sought to investigate further the role of SAMHD1 in macrophage proliferation and infection during development of SIVE and HIVE.

Materials and methods

Macaque tissue samples

Archived frozen and formalin-fixed paraffin-embedded monkey tissues including brain (frontal, temporal and occipital cortices and brainstem) and lymph nodes were prepared at the Tulane National Primate Research Center from 15 adult, male rhesus macaques (Macaca mulatta; Supplementary Table 1, http://links.lww.com/QAD/B241); four rhesus macaques were uninfected controls and the remaining 11 animals were infected intravenously with SIVmac251 or SIVmac239. Six of the monkeys had evidence of SIVE, defined by the presence of SIV-Gag p28 protein in the brain and the accumulation of macrophages and multinucleated giant cells (MNGCs). Animal studies where these animals were assigned were approved by the Tulane University Institutional Animal Care and Use Committee, and were carried out in accordance with the National Institutes of Health ‘Guide for the Care and Use of Laboratory Animals’, and the recommendations of the Weatherall report, ‘The use of nonhuman primates in research’.

Human brain tissues

Formalin-fixed, paraffin-embedded sections of parietal, temporal and occipital cortices were obtained from the Manhattan HIV Brain Bank, a member of the National NeuroAIDS Tissue Consortium. Brain tissues from multiple sclerosis cases that were used in Supplemental Digital Content were obtained from two (http://links.lww.com/QAD/B241) commercial sources (BioChain, Newark, California, USA; and Capital Bioscience, Gaithersburg, Maryland, USA). A total of three HIV cases, three HIV-1-positive cases without encephalitis and three seronegative controls that had been previously described elsewhere were examined [20,21].

Immunohistochemistry

Immunohistochemistry was performed, as previously described [21], using the antibodies listed in Supplementary Table 2, http://links.lww.com/QAD/B241. Detailed
experimental procedures are described in Supplemental Digital Content, http://links.lww.com/QAD/B241.

**Immunofluorescence microscopy**

Double-label or triple-label immunofluorescence was performed, as previously described [21], using the antibodies listed in Supplementary Table 2, http://links.lww.com/QAD/B241. Detailed experimental procedures are described in Supplemental Digital Content, http://links.lww.com/QAD/B241.

**Quantitative PCR for brain simian immunodeficiency virus DNA and RNA**

Quantification of SIV proviral DNA and 2-LTR DNA in brain tissues was performed using the TaqMan real-time qPCR method, as described previously [22,23]. Genomic DNAs from tissues were isolated by DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA) according to the manufacturer’s instructions, except for modification at the DNA elution step [24]. Total RNA was isolated from brain tissues by TRIZOL method. SIV RNA was quantified using multiplex one-step real-time quantitative reverse transcription PCR (RT-qPCR) method. Detailed experimental procedures are described in Supplemental Digital Content, http://links.lww.com/QAD/B241.

**Statistical analysis**

An unpaired t-test was used to determine significance in the quantification of SAMHD1-expressed and SAMHD1-expressing CD163+/SAMHD1+ cells (enumerated in at least 15 random fields of each tissue used at a magnification of 400x). The Pearson correlation coefficient was calculated to determine a linear relationship between levels of SIV DNA and RNA and the number of SAMHD1+ cells in the brain. A two-tailed P value was presented.

### Results

**SAMHD1 protein is strongly expressed in SIVmac251-infected macaque brains despite the presence of viral protein X**

Previous studies documented in-vitro SAMHD1 expression and Vpx-mediated SAMHD1 degradation in cultured HIV/SIV-target cells including monocytes, monocyte-derived macrophages (MDMs) and CD4+ T lymphocytes [5,6,9,10,25–28]. However, the cellular site of expression and regulation of SAMHD1 has not been extensively investigated in vivo. We sought to investigate SAMHD1 expression in macaque brain tissue, which is known to harbor SIV-infected macrophages. As a first step, we examined SAMHD1 protein expression, by immunohistochemistry in the frontal and/or temporal cortices and brainstem of uninfected control macaques (n = 3), SIV-infected macaques without encephalitis (SIVnoE, n = 5), and SIV-infected macaques with encephalitis (SIVE, n = 5). Three anti-SAMHD1 antibodies were tested, and all yielded similar reproducible staining (data not shown). Strong immunoreactivity appeared nuclear with weak cytoplasmic staining of some neurons. The uninfected control tissues showed little or no staining that was scattered and mainly associated with the central nervous system (CNS) vasculature (Fig. 1a). SIVnoE macaques had a higher frequency of SAMHD1 nuclear expression whenever compared with uninfected controls, but this too was scattered around the vessels (Fig. 1b). SIVE macaques expressed the most SAMHD1, with a high frequency of SAMHD1 seen in the perivascular space and within encephalitic lesions (Fig. 1c). This trend of increased expression with infection did not hold true in lymph nodes, where CD4+ T cells expressed SAMHD1 in both uninfected (Fig. 2a) and SIV-infected macaques (Fig. 2b) in similar amounts. Despite the characteristic reduction of CD4+ T cells during SIV infection, SAMHD1 expression pattern did not change. Thus, an increase in SAMHD1 expression during infection may be unique to the CNS.

**SAMHD1 is copresent with viral protein X in the central nervous system and lymph nodes of rhesus macaques infected with pathogenic SIVmac251**

This in-vivo observation that there was no apparent degradation of SAMHD1 protein in CD4+ lymphocytes of SIV-infected lymph nodes, in conjunction with a previous report of abundant Vpx protein present in the lymph node of SIV-infected macaques [29], runs contrary to previous in-vitro studies showing SIVmac Vpx-mediated depletion of SAMHD1 [5,6,9]. We, therefore, performed double-label immunofluorescent staining for Vpx and SAMHD1 on both SIV-infected lymph node (Fig. 2c) and encephalitic cortical brain tissue (Fig. 2d). Extensive co-expression of the retroviral restriction factor SAMHD1 and its (supposedly) counteracting viral protein Vpx was observed, especially in the cortex of the lymph nodes examined. Lesions within the SIVE brain tissue examined were noticeably dense in Vpx. This cytoplasmic staining surrounded clusters of SAMHD1+ nuclei (see inset in Fig. 2d), although scattered parenchymal SAMHD1+/Vpx+ cells were also observed. These observations suggest that, in multiple tissues, Vpx does not degrade SAMHD1; furthermore, at least the CNS, this lack of degradation is accompanied by increased SAMHD1 expression with encephalitis.

**SAMHD1 protein is localized to macrophages, microglia and endothelial cells in the brains of macaques with simian immunodeficiency virus encephalitis**

To better identify the cell types responsible for increased SAMHD1 protein expression during SIVE, we performed multilabel immunofluorescence staining of SAMHD1 together with various brain cell type-specific markers, including CD68, CD163, CNPase, GFAP, ...
Glut-1 and Iba-1. This labeling revealed that SAMHD1 is expressed on PVMs (Supplementary Fig. 1a, http://links.lww.com/QAD/B241), endothelial cells (Supplementary Fig. 1b, http://links.lww.com/QAD/B241) and microglia (Supplementary Fig. 1c, http://links.lww.com/QAD/B241), but not on astrocytes (Supplementary Fig. 1d, http://links.lww.com/QAD/B241) or oligodendrocytes (Supplementary Fig. 1e, http://links.lww.com/QAD/B241). A similar trend was observed in SIVnoE monkeys (data not shown). While making these observations, we noted that SAMHD1 expression on macrophages was more frequent than the other cell types explored. As macrophages are the major cell type in the brain infected by SIV, we undertook a quantitative examination of how the SAMHD1+ macrophage population changes with encephalitis. Representative photos from uninfected (d), SIVnoE (e) and SIVE (f) macaque frontal cortex demonstrate that both the total number of SAMHD1+ nuclei and the number of CD163+/SAMHD1+ macrophages are vastly increased in encephalitic animals compared with both uninfected and nonencephalitic animals. Enumeration of 15 random images from uninfected (n = 3), SIVnoE (n = 5) and SIVE (n = 5) animals confirmed that these increases were significant (g and h; unpaired t-test with two-tailed P values). CD163+/SAMHD1+ macrophages also positively correlated with CNS viral loads in SIVnoE and SIVE animals (i; Pearson correlation). Images d–f taken at 200× magnification. Scale bar in (d) also applies to (e) and (f). CNS, central nervous system; SAMHD1, sterile alpha motif and histidine-aspartate domain-containing protein 1; SIVE, simian immunodeficiency virus encephalitis. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Fig. 1. The number of SAMHD1+/CD163+ macrophages increases with encephalitis. Immunohistochemistry for SAMHD1 (DAB, brown) and a hematoxylin counterstain (blue) in uninfected (a), SIVnoE (b), and SIVE (c) revealed an increase in SAMHD1+ nuclei with SIVE in both the parenchyma and near brain vasculature. SAMHD1 expression was especially dense within encephalitic lesions. Images taken at 100× magnification. Scale bar in (a) also applies to (b) and (c). As macrophages are the major cell type in the brain infected by SIV, we undertook a quantitative examination of how the SAMHD1+ macrophage population changes with encephalitis. Representative photos from uninfected (d), SIVnoE (e) and SIVE (f) macaque frontal cortex demonstrate that both the total number of SAMHD1+ nuclei and the number of CD163+/SAMHD1+ macrophages are vastly increased in encephalitic animals compared with both uninfected and nonencephalitic animals. Enumeration of 15 random images from uninfected (n = 3), SIVnoE (n = 5) and SIVE (n = 5) animals confirmed that these increases were significant (g and h; unpaired t-test with two-tailed P values). CD163+/SAMHD1+ macrophages also positively correlated with CNS viral loads in SIVnoE and SIVE animals (i; Pearson correlation). Images d–f taken at 200× magnification. Scale bar in (d) also applies to (e) and (f). CNS, central nervous system; SAMHD1, sterile alpha motif and histidine-aspartate domain-containing protein 1; SIVE, simian immunodeficiency virus encephalitis. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.
correlation between the number of SAMHD1+CD163+ macrophages and CNS viral load (Fig. 1i) whereas interestingly, no significant correlation was found between CNS viral load and total SAMHD1+ cells (P > 0.05).

**Subpopulations of SAMHD1+ brain-resident macrophages are infected**

As the majority of SAMHD1-expressing cells were macrophages, we carefully examined if this cell type is infected by SIV. Triple-label immunofluorescence was performed on brain tissues from macaques with SIVE using antibodies against SAMHD1, CD163 and SIV Gag p28 protein. Our results indicated that SIVE animals demonstrated strong nuclear immunoreactivity for SAMHD1 in SIV antigen-expressing macrophages within encephalitic lesions (Fig. 3a) and in the perivascular space (Fig. 3b). SAMHD1-positive nuclei often clustered together in lesions. The scattered CD163+ parenchymal cells previously observed (Supplementary Fig. 2, http://links.lww.com/QAD/B241 and Fig. 2) were not immunoreactive for CD68, although weak parenchymal SIV p28 staining was infrequently observed and matched microglia morphologically. These cells were occasionally positive for SAMHD1.

**SAMHD1+-infected macrophages express proliferation markers in simian immunodeficiency virus encephalitic macaques and HIV-infected encephalitic humans**

We previously demonstrated the existence of a population of proliferating PVMs that are infected and contribute to lesion formation and establishment of the CNS viral reservoir [20]. As SAMHD1 normally depletes the dNTP pool, thus arresting cell-cycle progression, we were interested in whether this population of proliferating PVMs overlapped with the SAMHD1+-infected macrophage population described above. Co-expression of cycling markers such as proliferating cell nuclear antigen (PCNA) and Ki-67 with SAMHD1 would be evidence of a failure of SAMHD1 to successfully deplete the dNTP pool, and might point to its inactivation via phosphorylation. Indeed, triple-label immunofluorescent staining for Ki-67, SAMHD1, and either CD68 (Fig. 3c) or SIV p28 (Fig. 3d) revealed co-localization of SAMHD1 and Ki-67 (pink nuclei) within infected macrophages. Staining for PCNA, SAMHD1 and either SIVp28 (Fig. 3d) or CD68 (Fig. 3e) revealed a similar expression pattern. This is especially intriguing as it is well known that PCNA can directly interact with CDK2, which phosphorylates SAMHD1 at Thr592. Furthermore, a replication of this triple-label PCNA, SAMHD1 and CD68 staining in postmortem brain cortical tissue from HIV-1-infected patients with encephalitis showed similar co-localization of PCNA and SAMHD1 (pink nuclei) within CD68+ PVMs (Fig. 4c). This was in contrast to brain tissue from uninfected (Fig. 4a) or HIV-1-infected patients without encephalitis (Fig. 4b). In uninfected samples, SAMHD1 and CD68 immunoreactivity was low, and PCNA staining was very scattered and seemed to be mainly parenchymal. This pattern extended to nonencephalitic samples as well, with a slight increase in the amount of SAMHD1+ nuclei. Quantitative analysis was performed in the same manner as describe above for Fig. 1. Whenever comparing the number of CD68/SAMHD1 double-positive cells, the HIVE patients had significantly higher numbers of SAMHD1+ macrophages than the uninfected individuals (P = 0.0347). The above results suggest that SAMHD1 in infected CNS macrophages from both rhesus macaques and humans with encephalitis is present in an inactive form, allowing for proliferation of these viral antigen-expressing cells.

**SAMHD1 is phosphorylated at Thr592 in simian immunodeficiency virus-infected brain tissue**

Human SAMHD1 is inactivated via phosphorylation in MDMs by CDK2 at the highly conserved Thr592, becoming susceptible to infection. Brain tissue from eight monkeys, four SIVE and four SIVnoE, were probed for...
total SAMHD1 and Thr592 phosphorylated form of SAMHD1 (pSAMHD1), normalized against actin (Fig. 5). After probing for SAMHD1 with anti-SAMHD1 antibody, we found multiple bands possibly indicating the presence of both unphosphorylated and phosphorylated forms of SAMHD1 (Fig. 5a). To locate the band of pSAMHD1, we probed with a specific pSAMHD1 (Thr592) antibody. We found strong expression in all animals with increased expression found in SIVE animals (Fig. 5b). In addition to SAMHD1 and pSAMHD1, the brain sections were also probed for Vpx. Interestingly, we found Vpx expression alongside pSAMHD1 and SAMHD1, extending our previous finding via immunofluorescent microscopy of the copresence of SAMHD1 and Vpx (Fig. 1). This lends credence to the theory that, in vivo, Vpx does not participate in the degradation of SAMHD1.

**Discussion**

Retroviruses, such as HIV and SIV, rely on an enzyme, reverse transcriptase, to access dNTP substrates in order to convert single-stranded viral RNA to double stranded viral DNA [30–32]. However, these viruses must first overcome the SAMHD1 restriction blocks, which occur at the reverse transcription stage. SAMHD1 acts as a dNTPase, lowering the concentration of dNTP substrates below that which is required for reverse transcription [7,10]. Many ways to regulate this important protein in vitro are known including HIV-2/SIV Vpx-mediated degradation of SAMHD1. Using our rhesus macaque SIV model, we sought to determine whether the level of SIV infection of the brain corresponds to the level of SAMHD1 protein expression in adult rhesus macaque brain. Using immunohistochemistry and multilabel immunofluorescence microscopy for various markers for macrophages (CD68 and CD163), cell cycle (Ki-67 and PCNA), astrocytes (GFAP), microglia (Iba-1), oligodendrocytes (CNPase) and brain endothelial cells (GLUT1), along with SIV Gag protein (SIV p28), we were able to characterize the major cells types that express SAMHD1 in vivo, as well as determine whether these SAMHD1 cells were infected. From the double-label immunofluorescent staining and subsequent counting, we were able to show that macrophages are the major cell type that expresses SAMHD1 in a rhesus macaque SIV model. We demonstrate that SIV/HIV infection with encephalitis coincides with an increased number of
We also sought to investigate in-vivo evidence of the degradation of SAMHD1 by examining the level of SAMHD1 in chronically infected rhesus macaque lymph nodes, as well as any expression of a phosphorylated form of SAMHD1 in SIV-infected brain. We found no evidence in this study of SAMHD1 degradation in the presence of Vpx in vivo. It was long thought that the Vpx protein found in HIV-2 and SIV facilitated infection of macrophages by counteracting an unidentified restriction factor [33–37]. The absence of Vpx in HIV-1, in conjunction with that thought, has brought up a misconception that human tissue macrophages are nonpermissive to HIV-1. Once it was shown that SAMHD1 can be depleted in cells that expressed Vpx protein after either transfection with plasmids encoding Vpx or infection with virus-like particles containing Vpx, SAMHD1 was thought to be the long-sought Vpx-antagonized factor [5,6,14]. More recently, it was also shown that the presence of SIV Vpx correlated with reduced levels of SAMHD1 in CD4+ T cells both in vitro and in vivo during acute SIVmac239 infection [38]. Those findings contrast our own observations during pathogenic SIVmac251 infection: we found no in-vivo evidence that SAMHD1 was degraded in macrophages and CD4+ lymphocytes despite the presence of Vpx in the macaque tissues (Fig. 2). The differences in findings may be because of the specific cytokine milieu induced locally by SIV. For example, type I interferon has been shown to be responsible for the resistance of SAMHD1 in ex-vivo isolated dendritic cells to Vpx-mediated degradation [28]. This study showed that high levels of SAMHD1 protein in these cells cannot be counteracted by Vpx, which may demonstrate cell type-specific regulation of SAMHD1 expression. Additionally, Cenker et al. [39] showed that ex-vivo isolated brain microglia are highly permissive to HIV-1 infection despite high expression of SAMHD1. Our findings are consistent with a very recent report by Buchanan et al. in 2016 that SAMHD1 mRNA levels in the thalamus of rhesus macaques were upregulated, and demonstrated no association with decreasing viral load during in-vivo SIV infection.

In the current study, we examined the expression of SAMHD1 in brain tissues from patients with HIVE, progressive multifocal leukoencephalopathy (PML) and multiple sclerosis and have now found upregulation of SAMHD1 in HIVE as well as in PML and multiple sclerosis lesions (Supplementary Fig. 2, http://links.lww.
Therefore, it may not be HIV/SIV infection per se that drives upregulation of SAMHD1 in macrophages. It is entirely possible and likely that neuroinflammation (induced by HIV infection) is responsible for SAMHD1 upregulation. It was previously shown that interferons induce SAMHD1 expression in macrophages [40] and the type I interferon response plays a key role in exacerbation of neuroinflammation and contributes to progression of many neurodegenerative/demyelinating diseases including AGS and multiple sclerosis [41,42].

Because of persistent SAMHD1 expression in PVMs in the brain of SIVmac251-infected macaques, especially with encephalitis, we speculated that SAMHD1 exists as an inactive form. Human SAMHD1 is inactivated by its phosphorylation at the highly conserved Thr592 by CDKs [18,43]. MDMs became susceptible to HIV-1 infection when SAMHD1 is phosphorylated at Thr592 [18,44]. Interestingly, Yan et al. in 2015 showed that SAMHD1 is phosphorylated at Thr592 only during the S and G2 phases of the cell cycle in proliferating human monocytic cell lines. It was also demonstrated that proliferating (Ki67 + or MCM2 +) cells including in-vitro M2 macrophages (MDMs cultured in the presence of M-CSF at high concentrations) expressed the phosphorylated form of SAMHD1 that correlated with increased susceptibility to HIV-1 infection [18,19]. In this study, using a phosphospecific antibody against phosphorylated SAMHD1 at Thr592, pSAMHD1(Thr592), we found that the Thr592 phosphorylated (inactive) form was the dominant form of SAMHD1 in the brain of SIV-infected macaques, which corresponds to SIV infection of proliferating macrophages. Because of this increase in susceptibility in concordance with SAMHD1 phosphorylation, we believe that CDK6, an upstream regulator of CDK1/2, can be a potential therapeutic target in HIV infection [18]. CDK6 inhibition would result in downregulation of CDK1/2, thus a decrease in phosphorylation of SAMHD1 and possibly a decrease in susceptibility to infection.

Our results demonstrate that SIV infection coincides with an increased number of cycling, infected macrophages expressing SAMHD1. The presence of pSAMHD1 in the brain of SIVmac-infected macaques with encephalitis suggests that proliferation and SAMHD1 phosphorylation may predispose macrophages to become more susceptible to SIV infection. To our knowledge, the present study presents the first in-vivo evidence for SAMHD1 inactivation in brain during virus infection. Our study further suggests its role as a potential target for anti-HIV therapy.

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Conflicts of interest

There are no conflicts of interest.

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