SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates

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SAMHD1 restricts the infection of dendritic and other myeloid cells by human immunodeficiency virus type 1 (HIV-1), but in lentiviruses of the simian immunodeficiency virus of sooty mangabey (SIVsm)–HIV-2 lineage, SAMHD1 is counteracted by the virion-packaged accessory protein Vpx. Here we found that SAMHD1 restricted infection by hydrolyzing intracellular deoxynucleoside triphosphates (dNTPs), lowering their concentrations to below those required for the synthesis of the viral DNA by reverse transcriptase (RT). SAMHD1-mediated restriction was alleviated by the addition of exogenous deoxynucleosides. An HIV-1 with a mutant RT with low affinity for dNTPs was particularly sensitive to SAMHD1-mediated restriction. Vpx prevented the SAMHD1-mediated decrease in dNTP concentration and induced the degradation of human and rhesus macaque SAMHD1 but had no effect on mouse SAMHD1. Nucleotide-pool depletion could be a general mechanism for protecting cells from infectious agents that replicate through a DNA intermediate.

Myeloid cells such as dendritic cells (DCs) and monocyte-derived macrophages (MDMs) have an important role in orchestrating the innate and adaptive immune responses to virus infection. They also serve as natural targets of lentiviruses, including human immunodeficiency virus type 1 (HIV-1), that efficiently transmit the virus to activated CD4+ T cells and serve as a long-term reservoir that sustains ongoing virus infection. Infection of MDMs and DCs by HIV-1 is inefficient, which suggests that these cells may express a myeloid-specific restriction factor. Viruses of the HIV-2–simian immunodeficiency virus of sooty mangabeys (SIVsm) lineage express the accessory protein Vpx, which enhances their ability to infect myeloid cells and has been proposed to counteract the putative restriction factor[1]. The MDM- and DC-expressed target of Vpx has been identified as SAMHD1, a protein that contains a sterile α-motif and an HD domain[2,3].

The gene encoding human SAMHD1 was initially identified as an ortholog of a mouse gene induced by interferon-γ and in response to viral infection[4]. SAMHD1 has been proposed to act as a negative regulator of the innate immune response, analogous to TREX-1, a cytoplasmic exonuclease of single-stranded DNA that is thought to prevent the accumulation of virus and retrotransposon DNA to dampen innate immune responses[5]. Mutations in the genes encoding SAMHD1, TREX1 and RNase H2 are associated with Aicardi-Goutières syndrome, a rare early-onset inflammatory encephalopathy characterized by inappropriate immune activation and overproduction of interferon-α[6,7]. The SAMHD1 protein consists of an amino-terminal sterile α-motif domain and a central HD domain with putative nucleotidase and phosphodiesterase activities. Point mutations of the sequence encoding the conserved catalytic amino acid residues in the SAMHD1 HD domain inactivate its lentivirus-restriction activity[2,3,6,8]. SAMHD1 has weak homology with EF1143, an *Enterococcus fecalis* HD domain–containing bacterial nucleotide–metabolic enzyme[9], which further suggests that SAMHD1 has a role in nucleotide biochemistry. Homozygous deletions in the gene encoding SAMHD1 are associated with deletions of mitochondrial DNA, and mitochondrial diseases are often the result of mutations in genes that encode enzymes involved in the metabolism of deoxynucleoside triphosphates (dNTPs)[10–12]. The association of HD domains with phosphohydrolase activity[8,9] suggests that the antiviral activity of SAMHD1 could be mediated by an effect

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on dNTP metabolism. DCs have high expression of SAMHD1; MDMs have moderate SAMHD1 expression; and HIV-1-permissive $CD4^+$ T cells have only low SAMHD1 expression[3].

Cells differ in their dNTP content depending on cell type, differentiation and activation state and cell cycle[13]. The dNTP concentration in terminally differentiated, nonreplicating MDMs (20–40 nM) is less than 1% that of activated $CD4^+$ T cells (2–4 μM). Lentiviruses have evolved to replicate under conditions of low dNTP concentrations by virtue of a reverse transcriptase (RT) with a low Michaelis constant (K_m) for dNTPs[13,14]. Nevertheless, reverse transcription of HIV-1 proceeds more slowly in MDMs than in activated T cells[15,16]. The addition of deoxynucleosides to the culture medium accelerates the reverse transcription of HIV-1 in infected MDMs, which indicates that the intracellular dNTP pool is limiting in these cells[13,14,16,17]. Despite having an efficient RT, viruses of the HIV-2–SIVsm lineage depend on Vpx for the productive infection of monocytic cells[18,19].

Viruses of the HIV-2–SIVsm lineage express the accessory protein Vpx, which counteracts SAMHD1-mediated restriction[2,3]. Vpx is a small nuclear protein that is packaged in virions through a specific interaction with an amino acid motif in the p6 protein of the major virion structural precursor polyprotein Gag[20,21]. In the host cell, Vpx is associated with the multisubunit cullin 4A-ring E3 ubiquitin ligase CRL4. When expressed in a cell or introduced exogenously via virus-like particles (VLPs), Vpx binds to SAMHD1, and the associated CRL4 complex induces the ubiquitination and subsequent degradation of SAMHD1. HIV-1 does not express Vpx yet is highly sensitive to SAMHD1-mediated restriction[21,22].

Here we demonstrate that SAMHD1 lowered the concentration of intracellular dNTPs in myeloid cells to an amount that failed to support reverse transcription and thereby established a cellular state that was not permissive to lentiviral infection. Vpx induced degradation of SAMHD1, which resulted in a larger intracellular dNTP pool and restored permissiveness of the cell to infection. Although SIVmac Vpx counteracted human and rhesus SAMHD1, it was not active against the mouse homolog of SAMHD1.

RESULTS

A nucleotide phosphohydrolase that regulates dNTPs

Sequence comparison has shown that SAMHD1 has weak homology with EF1143 (ref. 9; **Supplementary Fig. 1**). That finding suggested that SAMHD1 might have a role in regulating intracellular dNTP pools in myeloid cells. To determine whether this was the case, we assessed the effect of knocking down SAMHD1 on the dNTP concentration in THP-1 human monocytic cells treated with phorbol 12-myristate 13-acetate (PMA). PMA-treated THP-1 cells model MDMs in their sensitivity to Vpx-containing VLPs and their lack of permissiveness to mutant SIV with deletion of the gene encoding Vpx (Δ*vpx* SIV)[3,23] (**Supplementary Fig. 2**). To quantify dNTPs, we used a single nucleotide–incorporation assay[13]. For this assay, an extract of cellular dNTPs is incubated with RT plus a double-stranded oligonucleotide in which one strand has a 5′ single-nucleotide overhang and the complementary strand is labeled at its 5′ end with ^{32}P. To quantify each of the nucleoside triphosphates, we used a set of four double-stranded oligonucleotides, each with a different overhanging nucleotide. We separated the products by gel electrophoresis and quantified the single nucleotide–extended primer on a phosphoimager. We found that in THP-1 cells in which SAMHD1 was stably knocked down by the transduction of short hairpin RNA (shRNA; **Fig. 1a**), the dNTP pool was larger than that of control cells that expressed shRNA with a scrambled sequence (**Fig. 1b**). Preincubation of PMA-treated THP-1 cells with Vpx-containing VLPs, which induces the degradation of SAMHD1, also resulted in a larger dNTP pool, mimicking the effect of knocking down SAMHD1 (**Fig. 1b**). This finding suggested that Vpx enhanced the dNTP supply by acting on SAMHD1.

To verify that SAMHD1 directly affected the pool of intracellular dNTPs, we assessed its ability to hydrolyze dNTPs *in vitro*. For this, we incubated *Eshcerichia coli*–produced recombinant SAMHD1 with each of the four deoxynucleotides separately, then analyzed the reaction products by thin-layer chromatography. We found that SAMHD1 hydrolyzed dGTP, liberating inorganic triphosphate (**Fig. 1c**), but was not active against the other three dNTPs (data not shown). The addition of unlabeled dGTP, however, stimulated the hydrolase activity of SAMHD1 on the other three dNTPs (**Fig. 1c**). Analysis of the reaction products showed that the three phosphates were removed in a single step, resulting in free inorganic triphosphate (**Supplementary Fig. 3**). The findings that SAMHD1 had dNTP-phosphohydrolase activity and that diminishing its abundance in the cell resulted in a greater abundance of dNTPs suggested that it directly regulated the intracellular concentration of dNTPs.

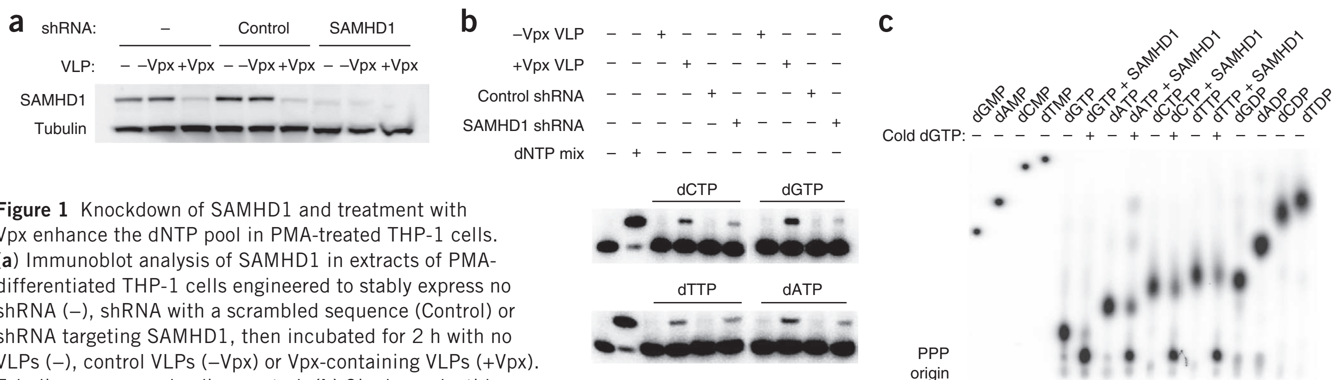

Figure 1 Knockdown of SAMHD1 and treatment with Vpx enhance the dNTP pool in PMA-treated THP-1 cells. (**a**) Immunoblot analysis of SAMHD1 in extracts of PMA-differentiated THP-1 cells engineered to stably express no shRNA (–), shRNA with a scrambled sequence (Control) or shRNA targeting SAMHD1, then incubated for 2 h with no VLPs (–), control VLPs (–Vpx) or Vpx-containing VLPs (+Vpx). Tubulin serves as a loading control. (**b**) Single nucleotide–incorporation assay[13] of dNTPs in cells treated as in **a**. (**c**) Thin-layer chromatography analysis of the dNTP-triphosphohydrolase activity of recombinant wild-type SAMHD1 (1 μM; + SAMHD1) incubated with each dNTP or dNDP (above lanes), 1 μCi of the corresponding [α-^{32}P]dNTP, with (+) or without (–) unlabeled (Cold) dGTP (200 μM); unlabeled standards were visualized by ultraviolet shadowing and [α-^{32}P]-labeled nucleotides were visualized with a phosphorimager. Lanes without '+ SAMHD1' represent analysis of dNTP without SAMHD1. PPP, triphosphate. Data are from one experiment representative of three.

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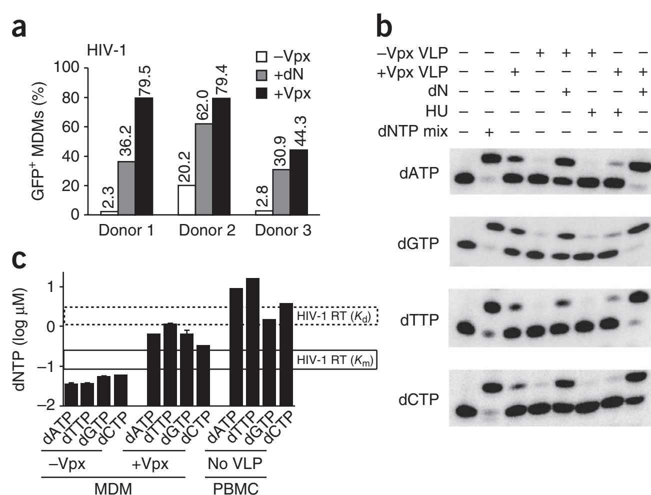

Figure 2 Vpx increases the intracellular pool of dNTPs in MDMs. (**a**) Quantification of GFP^+ cells by flow cytometry of MDMs obtained from healthy human donors ($n = 3$), preincubated with Vpx-containing or control VLPs or with 2.5 mM deoxynucleosides (+dN), then infected with HIV-GFP reporter virus (multiplicity of infection, 1). Numbers above bars indicate percent GFP^+ cells. (**b**) Single nucleotide–incorporation analysis of dNTPs in MDMs treated with VLPs, 2 mM deoxynucleosides or 2 mM hydroxyurea (HU). (**c**) Quantification of dNTPs in PBMCs and MDMs from healthy donors; solid lines indicate K_m of HIV-1 RT, and dashed lines indicate K_d (dissociation constant) of HIV-1 RT[28]. Data are representative of three experiments (**a**), two experiments with one donor (**b**) or one experiment with two donors (**c**).

SAMHD1 regulates the dNTP pool in MDMs

Next we assessed the role of SAMHD1 in regulating the intracellular pool of dNTPs in primary MDMs. Exogenous deoxynucleosides are taken up by the cell and are directly converted into dNTPs through the salvage pathway of dNTP synthesis, which results in a larger intracellular dNTP pool[24–26]. To determine whether increasing the abundance of intracellular dNTPs would relieve the block to HIV-1 infection, we purified MDMs from the peripheral blood mononuclear cells (PBMCs) of healthy human donors and treated the MDMs with deoxynucleosides. We found that treatment with deoxynucleosides or with Vpx-containing VLPs enhanced HIV-1 infection (**Fig. 2a**). Treatment with deoxynucleosides was less effective than was treatment with Vpx-containing VLPs, probably because cells treated with deoxynucleosides continue to hydrolyze the dNTPs, whereas in cells exposed to Vpx-containing VLPs, SAMHD1 is degraded, which prevents hydrolysis of dNTPs.

To determine the effect of the treatment with deoxynucleosides or Vpx on each of the four dNTPs, we measured the intracellular concentrations of dNTPs by the single nucleotide–incorporation assay (**Fig. 2b**). We found that treatment with Vpx-containing VLPs or with deoxynucleosides resulted in 5- to 33-fold higher concentrations of the four dNTPs in MDMs (**Fig. 2b**), depending on the nucleotide (**Supplementary Table 1**). To further assess the role of SAMHD1 in regulating dNTP concentrations in MDMs, we treated the cells with hydroxyurea. Hydroxyurea is an inhibitor of ribonucleotide reductase, the enzyme that converts rNDPs to dNDPs and thus prevents the synthesis of dNTPs[27]. We found that hydroxyurea blocked the Vpx-mediated increase in the dNTP supply (**Fig. 2b**). These results suggested that the production of dNTPs by ribonucleotide reductase was required for Vpx to be effective. In sum, these results further supported the proposal that SAMHD1 has a role in regulating the intracellular pool of dNTP.

Quantification of the individual dNTPs showed that treatment of the MDMs with Vpx-containing VLPs increased the concentration of dNTPs in these cells to about 20% that of activated PBMCs (**Fig. 2c**). This higher dNTP concentration was above the K_m of HIV-1 RT but below the dissociation constant of HIV-1 RT[28]. This indicated that in MDMs, SAMHD1 activity resulted in a dNTP concentration that was suboptimal for viral DNA synthesis. By inhibiting SAMHD1, Vpx increased the concentration of dNTPs to an amount that was sufficient to accelerate the rate of viral DNA synthesis by RT.

SAMHD1 restricts HIV-1 by depleting the dNTP pool

To determine whether SAMHD1 was sufficient to decrease the intracellular dNTP concentration, we used U937 cells, a monocytoid line that does not express endogenous SAMHD1. We transduced these cells with lentiviral vectors with high or moderate expression of human SAMHD1 or with expression of mouse SAMHD1 isoform 1 or 2, rhesus macaque SAMHD1, or catalytically inactive human SAMHD1 in which the consensus histidine and aspartate residues were substituted with alanine (HD-AA), and established matched control lentiviral vector cell lines in parallel (**Fig. 3a**). Immunoblot analysis of these transduced U937 cells showed that SAMHD1 expression was dependent on treatment with PMA (**Fig. 3a**). The PMA dependence was due to more transcription of the cytomegalovirus promoter of the lentiviral vector that most likely resulted from the upregulation of host transcription factors (data not shown). In the absence of PMA, the dNTP concentration in these cells was relatively unaffected by the various SAMDH1 constructs expressed by lentiviral vectors (**Fig. 3b** and **Supplementary Fig. 4a**). However, after treatment of the cells with PMA, expression of SAMHD1 was accompanied by a considerable decrease in the dNTP pool (**Fig. 3b** and **Supplementary Fig. 4b**). High or moderate expression of human SAMHD1 by the transduced cells resulted in dNTP concentrations that were 2.4% and 5% that of the cells transduced with control lentiviral vector, respectively. In transduced cell lines with the highest expression of mouse SAMHD1 (isoform 2), the dNTP concentration was 1.8% that of cells transduced with control lentiviral vector. The HD-AA mutant human SAMHD1 had no effect on the dNTP concentration, which indicated that the catalytic activity of SAMHD1 was required for the decrease in dNTP concentration. Rhesus macaque SAMHD1 and mouse SAMHD1 isoform 1, which had somewhat lower expression than human SAMHD1, caused a less pronounced decrease in dNTP concentration. We detected slightly lower dNTP concentrations in the U937 cell line that expressed mouse SAMHD1 isoform 2, even in the absence of PMA treatment (**Fig. 3a**), which suggested that the expression of even trace amounts of SAMHD1 was sufficient to detectably affect dNTP concentrations.

U937 cells are highly susceptible to infection with HIV-1 (somewhat more susceptible than THP-1 cells treated with Vpx-containing VLPs or stably depleted of SAMHD1 by transduction of shRNA; **Supplementary Fig. 5**). To assess the antiviral activity of the transduced SAMHD1 proteins, we infected the PMA-treated U937 cell lines with replication-defective wild-type or Δ*vpx* SIVmac239 reporter virus. These viruses express heat-stable antigen (mouse CD24) and are limited to a single cycle of replication. Quantification of reporter virus–infected $CD24^+$ cells by flow cytometry confirmed the antiviral activity of the transduced SAMHD1 (**Fig. 3c**). This antiviral activity was dependent on the catalytic activity of SAMHD1, as shown by the lack of antiviral activity of the HD-AA mutant human SAMHD1. Vpx counteracted the restriction mediated by human and rhesus

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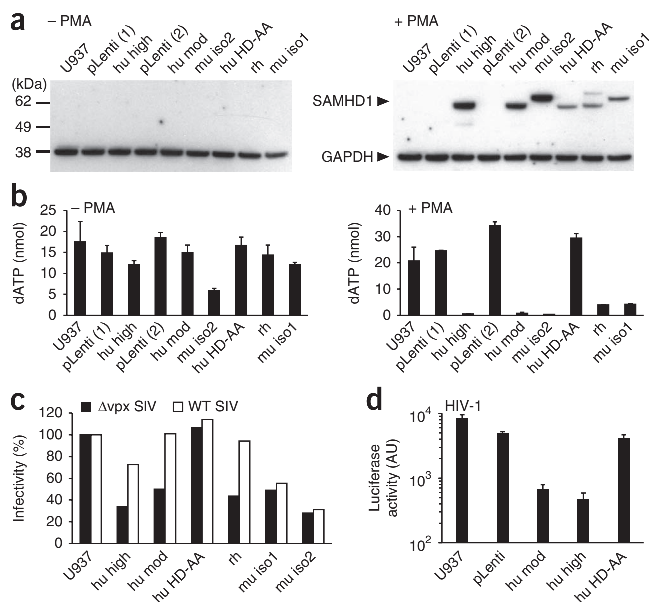

Figure 3 SAMHD1 diminishes the intracellular dNTP pool, and human and rhesus SAMHD1, but not mouse SAMHD1 homologs, are counteracted by Vpx. (**a**) Immunoblot analysis of SAMHD1 in U937 cells transduced with empty vector (pLenti (1) and pLenti (2)), vector with high (hu high) or moderate (hu mod) expression of human SAMHD1, or vector expressing mouse isoform 1 (mu iso1) or 2 (mu iso2) of SAMHD1, the HD-AA mutant human SAMHD1 (hu HD-AA), or rhesus macaque SAMHD1 (rh), then left untreated (left) or treated with PMA (right). GAPDH (glyceraldehyde phosphate dehydrogenase) serves as a loading control. Left margin, molecular size markers, in kilodaltons (kDa). (**b**) Quantification of dNTPs in U937 cells treated as in **a**. (**c**) Flow cytometry of U937 cells transduced and treated with PMA as in **a**, then infected with Δ*vpx* or wild-type (WT) SIVmac239 reporter virus and stained with monoclonal antibody to mouse CD24. (**d**) Luciferase activity of PMA-treated U937 cells treated as in **a**, then infected for 2 d with an HIV-1 luciferase reporter virus. AU, arbitrary units. Data are representative of three experiments (**a**,**c**) or two experiments (**b**,**d**; average and s.d. of duplicates (**b**) or triplicates (**d**)).

SAMHD1. In contrast, mouse SAMHD1 was resistant to Vpx. Consistent with that finding, Vpx-containing VLPs induced the degradation of human and rhesus SAMHD1 but had no effect on the mouse protein (data not shown). Human, mouse and rhesus SAMHD1 also restricted HIV-1 in transduced U937 cells, and this activity was dependent on the catalytic activity of the enzyme (**Fig. 3d** and data not shown).

HIV-1 with substitution of isoleucine for valine at position 148 of RT (V148I mutant RT) replicates in primary CD4+ T cells but not in MDMs[29]. The inability of this virus to replicate in MDMs is due to the higher K_m and lower binding affinity of the mutant RT for dNTPs, coupled with the low concentration of dNTPs in these cells[13]. We sought to determine whether degradation of SAMHD1 by Vpx would restore the ability of HIV-1 with V148I mutant RT to infect the host cell. To address this, we added Vpx-containing VLPs and control VLPs that lack Vpx to MDMs isolated from human donors, infected these cells with HIV reporter virus containing a cloned gene encoding green fluorescent protein (HIV-GFP), expressing either wild-type RT or V148I mutant RT, and quantified the GFP+ cells by flow cytometry. HIV-GFP expressing V148I mutant RT was less infectious than wild-type HIV-GFP, and Vpx enhanced the infectivity of the wild-type and mutant viruses (**Fig. 4a**). However, in the presence of Vpx, the difference between the wild-type and mutant viruses in their infectivity was less (**Fig. 4b**). Although this difference was small, it further supported the hypothesis that SAMHD1 limited the ability of HIV-1 to productively infect MDMs. These findings suggested that SAMHD1 could be more restrictive to viruses in which the RT has a lower affinity for dNTPs, as may be the case for primary viruses *in vivo*.

Extracellular nucleosides compensate for Vpx in MDMs

We next determined whether providing a source of dNTPs to the cells would relieve the block to the replication of Δ*vpx* SIV, a virus that is unable to degrade SAMHD1. We exposed MDMs from healthy human donors to increasing concentrations of deoxynucleosides, together with Vpx-containing VLPs or control VLPs, and then infected the cells with a Δ*vpx* SIVsm reporter virus that expresses GFP (**Fig. 5**). As the deoxynucleoside concentration increased, the infectivity of Δ*vpx* SIVsm in cells treated with control VLPs approached the infectivity of the virus in cells treated with Vpx-containing VLPs. This finding further supported the hypothesis that SAMHD1 restricts lentivirus replication by diminishing the intracellular pool of dNTPs and that the role of Vpx is to increase the intracellular pool of dNTP by inducing the degradation of SAMHD1.

The effect of SAMHD1 depends on the cellular context

Activated primary CD4+ T cells express only a small amount of SAMHD1 and are fully susceptible to HIV-1 and Δ*vpx* SIV[3]. Treatment of these cells with Vpx-containing VLPs had only a slight effect on dNTP concentrations (**Supplementary Fig. 6**), which indicated that SAMHD1 had a minor role in controlling dNTP concentration in these cells. To determine whether ectopic expression of SAMHD1 restricted HIV-1 replication in T cells, we stably expressed SAMHD1 in the CD4+ human lymphoma T cell line SupT1 by retroviral vector transduction. Overexpression of SAMHD1 had no effect on HIV-1 infection relative to the infection of control SupT1 cells that were not transduced (**Supplementary Fig. 7**). However, when we inhibited ribonucleotide reductase by treating cells with hydroxyurea, SupT1 cells that expressed SAMHD1 were more resistant to infection than were control cells that did not express SAMHD1 (**Supplementary Fig. 7**). These results suggested that the high rate of dNTP production in the T cells rapidly replenishes the dNTPs that are hydrolyzed by SAMHD1, limiting its antiviral activity in these cells.

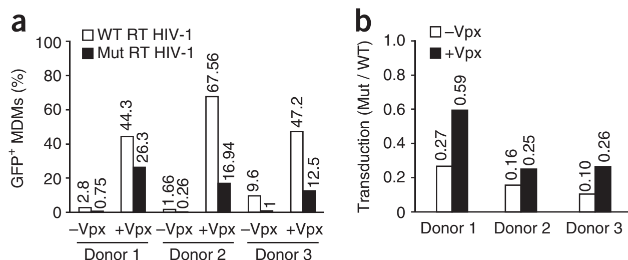

Figure 4 Vpx restores the infectivity of HIV-1 expressing mutant RT with lower affinity for dNTPs. (**a**) Quantification of GFP+ cells by flow cytometry of MDMs obtained from healthy human donors ($n = 3$), preincubated with Vpx-containing or control VLPs, then infected for 4 d with wild-type or V148I mutant (Mut) HIV-GFP (multiplicity of infection, 1). Numbers above bars indicate percent GFP+ cells. (**b**) Efficiency of infection in the presence and absence of Vpx as in **a**, presented as the ratio of mutant virus to wild-type virus (a ratio of 1 indicates equivalent infection efficiency). Numbers above bars indicate ratio. $P < 0.05$, wild-type versus mutant (two tailed Mann-Whitney test). Data are representative of three experiments.

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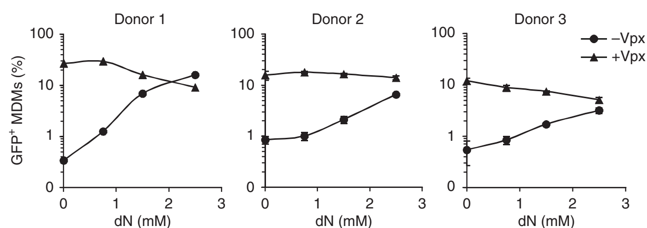

Figure 5 The salvage pathway of dNTP synthesis partially restores the infectivity of Δ*vpx* SIVmac in MDMs. Frequency of GFP+ MDMs among MDMs obtained from healthy human donors ($n = 3$), preincubated with Vpx-containing or control VLPs and infected with Δ*vpx* SIVsm reporter virus that expresses GFP (multiplicity of infection, 1), followed by stimulation of the salvage pathway of dNTP synthesis by the addition of 0.75, 1.5 or 2.5 mM deoxynucleosides to the medium (from 2 h before infection to 20 h after infection), assessed by flow cytometry 4 d after infection. Data are representative of two experiments.

DISCUSSION

Our findings have suggested that the main mechanism by which SAMHD1 restricts lentivirus infection of myeloid cells is by decreasing the concentration of dNTPs to below the K_m of RT, thereby blocking reverse transcription. It has been reported that recombinant SAMHD1 is a dNTP triphosphohydrolase[30,31], a finding that we have confirmed here and that suggests SAMHD1 directly regulates the intracellular dNTP pool. HIV-2–SIVsm lentiviruses counteract SAMHD1 via Vpx brought into the target cell by the incoming virion. Vpx induced the proteosomal degradation of SAMHD1 in the target cells, which resulted in higher intracellular dNTP concentrations through new synthesis.

SAMHD1-mediated depletion of the intracellular dNTP pool provides a means by which the cell establishes an antiviral state without the need for an inhibitor that interacts directly with a specific viral component. Thus, this mechanism may serve to restrict a diverse range of retroviruses. In addition, this mechanism explains how SAMHD1 can block reverse transcription, which occurs in the cytoplasm, even though it is localized to the nucleus, a spatially separated cellular compartment. Furthermore, it provides a rationale for why SAMHD1 restriction operates only in terminally differentiated and nondividing cells such as MDMs and DCs. Those cells, in contrast to dividing cells, do not need to maintain a high concentration of dNTPs and therefore are not harmed by the catalytic activity of SAMHD1. Expression of a transduced gene encoding SAMHD1 in actively dividing cells had little effect on dNTP concentrations and did not restrict infection. Moreover, we detected endogenous SAMHD1 expression in activated CD4+ T cells, yet these cells maintained a high concentration of dNTPs (data not shown). These findings suggest that either the catalytic activity of the enzyme is regulated in actively replicating cells or the rate of dNTP production is sufficient to replenish the dNTP pool. Consistent with the latter possibility, treatment of cells with hydroxyurea to block dNTP synthesis demonstrated the ability of SAMHD1 to restrict infection. It is notable that Vpx packaged in the incoming virion was able to induce enough degradation of SAMHD1 to substantially increase the dNTP concentration in the target cell. SAMHD1 degradation has been demonstrated before through the use of Vpx-containing VLPs, but here we found that it also occurred when Vpx was packaged in the incoming virion.

HIV-1 is highly sensitive to SAMHD1-mediated restriction yet lacks Vpx. It is possible that *in vivo*, HIV-1 replication is sustained mainly by T cells and thus there is no selective advantage in maintaining the open reading frame of the gene encoding Vpx. Alternatively, SAMHD1 could provide a selective advantage to HIV-1 by limiting the ability of the virus to infect myeloid cells. These cells contain sensors for cytoplasmic DNA as well as a cryptic sensor that detects newly synthesized viral proteins[32]. It has been suggested that the lack of Vpx serves as a strategy by which the virus avoids activating cytoplasmic sensors and thereby limits the induction of inflammatory cytokines[32]. The identity of the viral protein sensor is not known and is probably not SAMHD1 itself. Nevertheless, SAMHD1 is interferon inducible[33], and the cryptic sensor could serve to induce SAMHD1 expression in nearby cells.

The role of the Vpx-related lentiviral accessory protein Vpr *in vivo* remains poorly understood. Like Vpx, Vpr is packaged in virions and interacts with the E3 ubiquitin ligase CRL4 (refs. 34,35) but has only a modest effect on MDM and DC infection. Although SIVmac lacking Vpr or Vpx is only slightly less pathogenic than wild-type SIVmac in rhesus macaques, SIVmac lacking both Vpx and Vpr is highly attenuated, which suggests an overlap in function[36]. Unlike Vpx, SIVmac Vpr did not interact detectably with SAMHD1 and did not affect the concentration of dNTPs in cultured MDMs (data not shown). Nevertheless, it remains possible that Vpr influences a nucleotide-metabolic pathway that is not active under standard culture conditions and that therefore its effect is not detected. It is also possible that for some SIV isolates, Vpr interacts with SAMHD1 and has Vpx-like function.

In addition to its role in counteracting SAMHD1, Vpx is reported to bind to the cytidine deaminase APOBEC3A[37], and it has been suggested that Vpx has a dual role in counteracting the two virus-restriction factors. This could be explained if the higher concentration of dNTPs that results from Vpx-induced degradation of SAMHD1 allows more rapid reverse transcription. The higher rate of viral DNA synthesis could then protect the transcript from deamination by APOBEC3A.

To replicate their genomes, DNA viruses and viruses that replicate through a DNA intermediate need to access dNTPs and have evolved various strategies to ensure their availability. Small DNA viruses 'preferentially' infect mitotic cells; adenoviruses, polyomaviruses and papillomaviruses encode proteins that drive quiescent cells into S phase[38]; and herpesviruses and poxviruses encode an ribonucleotide reductase that converts NTPs to dNTPs[38,39]. For retroviruses, the limited supply of dNTPs in nondividing and quiescent cells presents a particular challenge[24,40]. The requirement for high concentrations of dNTPs by gamma retroviruses impairs the reverse transcription of these viruses in quiescent and nondividing cells[41,42]. Lentiviruses have adapted to this problem in part by developing an RT with a low K_m for dNTP[13,14,16,17]. Despite their efficient RT, HIV-1 and HIV-2 remain sensitive to the low concentration of dNTPs in the cytoplasm of their natural target cells. HIV-1 seemed to be more sensitive to SAMHD1-mediated restriction than was Δ*vpx* SIV and was more sensitive to the addition of deoxynucleosides to the culture medium (data not shown). HIV-1 in which RT was altered to decrease its affinity for dNTP was more sensitive to SAMHD1. These findings suggest that HIV-1 isolates may vary in their sensitivity to SAMHD1 and that variability in intracellular dNTP concentrations as a result of cellular activation state and genetic polymorphism could influence HIV-1 replication.

By depleting the pool of available dNTPs, SAMHD1 effectively starves the virus of a building block that is central to its replication strategy. The use of Vpx to destroy SAMHD1 represents a notable strategy developed by lentiviruses to thwart cellular antiviral defenses. These findings bring to light a previously unknown mechanism of innate immunity and raise the question of whether the pharmacological alteration of intracellular dNTP pools might be

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a therapeutic approach to treating virus infection. The implications of depleting the nucleotide pool as a host-defense mechanism are potentially far-reaching.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureimmunology/.

Note: Supplementary information is available on the Nature Immunology website.

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AUTHOR CONTRIBUTIONS

H.L., W.D., H.H., M.Ben., N.R.L., N.B., C.T., B.K. and F.M.-G. conceived of and did the experiments; H.L., M.Ben., C.T., B.K., N.R.L. and F.M.-G. wrote the paper; and D.A., E.C.L., L.D., C.M., T.G., G.P., N.L., M.Ber., B.C. and S.P. designed and did some of the experiments.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Plasmid construction. Hemagglutinin-tagged SAMHD1 constructs were generated by PCR with primers containing BamHI and XhoI cleavage sites. PCR products were cloned into the BamHI and SalI sites of the pLenti-puro lentiviral vector (Cell Biolabs). Human, rhesus and mouse SAMHD1 was amplified from cDNA generated by reverse transcription of RNA from THP-1 cells, rhesus PBMCs and mouse bone marrow–derived DCs with Transcriptor reverse transcriptase (Roche).

Cells. PBMCs from the blood of anonymous donors (obtained in accordance with the ethical guidelines of the Institut Cochin) were prepared by Ficoll density-gradient separation and monocytes were isolated by positive selection on CD14 magnetic microbeads (Miltenyi Biotec), followed by culture in BD Primaria flasks with R10 medium (RPMI-1640 GlutaMAX-I, 10 mM HEPES, pH 7.2, 1 mM sodium pyruvate, 1% (vol/vol) nonessential amino acids and 10% (vol/vol) heat-inactivated FCS) and antibiotics. MDMs were obtained from monocytes by culture of the cells for 7–9 d with granulocyte-macrophage colony-stimulating factor (10 ng/ml) and macrophage colony-stimulating factor (20 ng/ml). THP-1 cells expressing shRNA with scrambled sequence or shRNA targeting SAMHD1 have been described[3].

For the establishment of U937 cells that stably expressed SAMHD1, lentiviral vector stocks were generated by calcium-phosphate transfection of 293T human embryonic kidney cells with pLenti vectors for human, rhesus or mouse SAMHD1 and expression plasmids pRSV-Rev, pMDG gag-pol and VSV-G. U937 cells were 'spin-infected' with lentiviral vectors, and transduced cells were selected in puromycin (1 µg/ml).

Immunoblot analysis. U937 cells lines stably expressing SAMHD1 were differentiated for 20 h with PMA (30 ng/ml) and lysed in radioimmunoprecipitation buffer containing protease inhibitor, then normalized for protein content. Whole-cell lysates containing 10 µg total protein were separated by SDS-PAGE. Proteins were transferred to a polyvinylidene difluoride membrane and probed with a mixture of monoclonal antibody to hemagglutinin (HA.11; Covance) and monoclonal antibody to GAPDH (Ambion), followed by horseradish peroxidase–conjugated goat antibody to mouse immunoglobulin (PA1-85999; Pierce). Proteins were visualized with Super Signal West Pico chemiluminescent substrate (ThermoScientific). SAMHD1 and tubulin were detected with antibodies ab67820 (Abcam) and T9026 (Sigma), respectively.

Viruses, VLPs and infection. Viruses were produced in 293T cells cotransfected with reporter virus plasmid and VSV-G by the calcium-phosphate method. Wild-type and V148I mutant HIV-GFP reporter viruses were produced by cotransfection of 293T cells with pRRLsin.eGFP and pCMVΔ8.2 or pCMVΔ8.2 V148I and VSV-G plasmid[43–45]. The Δ*vpx* SIVmac virus was produced by cotransfection of cells with pSIV3+ Δ*vpx* and the minimal SIV genome pGAE 1.0, in which GFP expression is driven by the cytomegalovirus promoter[46–48]. Vpx-containing and control VLPs were produced by transfection of 293T cells with pSIV3$^+$ (VLPs Vpx$^+$) or pSIV3$^+$ Δ*vpx* (VLPs Vpx$^-$) in the absence of a viral genome[46–48]. Culture supernatants were collected and filtered 24–48 h after transfection and were concentrated by polyethylene-glycol precipitation.

Cells were infected as described before for infection of DCs[46]. Cells were preincubated with VLPs for 2 h before infection. The minimal amount of VLPs (Vpx$^+$) required for maximal helper effect for HIV-1 infection was determined by titration. Equivalent amounts of Vpx-containing and control VLPs were normalized by p27 capsid protein by enzyme-linked immunosorbent assay. After preincubation, cells were infected for 2 h with a GFP reporter virus at a multiplicity of infection of 1 (predetermined by titration on HeLa human cervical cancer cells). Infected cells were treated with deoxynucleosides, which consisted of a mixture of dA (D8668), dC (D0776), dG (D0901) and dT (T1895; all from Sigma-Aldrich) or with 2 mM hydroxyurea. At 4 d after infection, GFP$^+$ cells were quantified by flow cytometry.

SIVmac239 reporter viruses lacking the envelope protein alone or lacking both envelope protein and Vpx were produced as VSV-G pseudotypes. Viruses were titered on 293T cells by determination of the number of CD24$^+$ cells by flow cytometry. U937 cells were differentiated for 20 h in PMA (30 ng/ml) and then infected with reporter virus at a multiplicity of infection of 1. Infected cells were quantified by flow cytometry. For luciferase reporter assays, cells were infected with 10 ng VSV-G pseudotyped NL.LucE$^-$R$^-$ (ref. 49). Luciferase activity was measured after 48 h with a Steadylite Luminescence Reporter Gene Assay (PerkinElmer). P values were determined with the two-tailed Mann-Whitney test.

Quantification of whole-cell dNTP pools. The dNTPs were quantified as described[13]. Cells were washed with cold PBS and were lysed for 30 min in 65% (vol/vol) aqueous methanol. Lysates were heated to 95 °C, clarified for 3 min by centrifugation at 16,000*g*, dried under vacuum and resuspended in double-distilled H_2O. An 18-nucleotide primer (5′-GTCCCTGTTCGGGCGCCA-3′) labeled at the 5′ end with ^{32}P was annealed to four different 19-nucleotide templates (5′-NTGGCGCCCGAACAGGGAC-3′, where 'N' represents nucleotide variation at the 5′ end) at a primer-to-template ratio of 1:2. Each reaction volume of 20 µl contained 200 fM template and primer, 2 µl of 0.5 mM dNTP mix or cell extract, 25 mM Tris-HCl, pH 8.0, 2 mM dithiothreitol, 100 mM KCl, 5 mM $MgCl_2$ and 10 µM oligo(dT). Reactions were incubated for 5 min at 37 °C in the presence of excess HIV-1 RT, then were terminated by the addition of 10 µl stop dye (40 mM EDTA and 99% (vol/vol) formamide) and denaturation for 5 min at 95 °C. Extended and unextended products were resolved by 16% urea-PAGE and analyzed on a phosphorimager. The frequency of extended primers was determined with QuantityOne software. The dNTP content of a standard dilution of each sample was determined and the sample volume was then adjusted to obtain a signal within the linear range of the assay. The addition of pure dNTPs to dNTPs prepared from SAMHD1-expressing cells showed that the results were not influenced by the presence of inhibitors in the cellular dNTP preparation (data not shown).

Assay of dNTPase activity. Recombinant SAMHD1 (1 µM) was incubated for 3 h at 37 °C in 50 mM Tris-HCl, pH 8.0, 50 mM KCl, 5 mM MgCl2, 0.1% (vol/vol) Triton X-100, 200 µM dNTP, 1 µCi of the corresponding [γ-^{32}P]dNTP. Where indicated, 200 µM unlabeled dGTP was added to the reaction. Reactions were stopped by incubation for 5 min at 70 °C. Samples were separated on polyethyleneimine cellulose thin-layer chromatography plates (Macherey Nagel) with a mobile phase of 0.8 M LiCl. After plates were dried, unlabeled standards were visualized by ultraviolet shadowing and [γ-^{32}P]-labeled nucleotides were detected with a phosphorimager.

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Erratum: SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates

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In the version of this article initially published, the number for Baek Kim's second affiliation is incorrect in the author list. The correct number is 10. The error has been corrected in the HTML and PDF versions of the article.

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