SAMHD1 does it again, now in resting T cells

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A long-standing question in the HIV field is why HIV-1 fails to replicate in resting CD4+ T cells. A new study shows that host deoxynucleoside triphosphate triphosphohydrolase (dNTPase) sterile α motif and histidine/aspartic domain–containing protein 1 (SAMHD1), previously shown to block HIV infection in myeloid cells, also restricts HIV replication in resting CD4+ T cells by hydrolyzing dNTPs, which are needed for reverse transcription of the virus (pages 1682–1687).

One of the puzzles of HIV-1 biology is why the virus barely replicates in resting CD4+ T lymphocytes, whereas activated CD4+ T lymphocytes support robust HIV-1 replication. In HIV-infected people, very few T lymphocytes in the peripheral blood or lymph node are actively infected (even in the absence of antiviral treatment), and viral replication is mostly restricted to activated CD4+ T cells. In resting CD4+ T cells, reverse transcription, which copies the viral genomic RNA to cDNA soon after viral entry before nuclear import and chromosomal integration, is very inefficient, and other barriers to viral production appear at later steps in the viral life cycle[1,2].

The study by Baldauf *et al.*[3] in this issue *of Nature Medicine* provides a simple and elegant explanation for HIV-1 restriction in resting T cells (**Fig. 1**). The authors identify SAMHD1, a dNTPase that removes the triphosphate from dNTPs, as a host restriction factor for HIV-1 in resting CD4+ T cells.

Mutations in *SAMHD1* are associated with Aicardi-Goutières syndrome (AGS), an autoinflammatory genetic brain disorder of newborns that mimics congenital infection and is characterized by elevated amounts of type I interferons[4]. SAMHD1 was identified in 2011 as the long-sought restriction factor for productive infection by HIV of myeloid dendritic cells, monocytes and macrophages[5,6]. SAMHD1 limits HIV-1 replication in these cells by depleting intracellular dNTPs, thus blocking HIV DNA synthesis by reverse transcription[7]. High dNTP concentrations are not needed in nondividing cells and are kept low; myeloid cells have about 1% of the concentration of dNTPs in activated T cells. Although HIV-1 reverse transcriptase very efficiently uses dNTPs, the dNTP concentration in myeloid cells is rate limiting. The small lentiviral accessory protein Vpx (from SIV or HIV-2, which is missing in HIV-1) targets SAMHD1 for ubiquitination and degradation. Supplying Vpx or knocking down SAMHD1 at the time of HIV-1 infection enhances dNTP concentrations within hours, promotes reverse transcription and drastically improves HIV-1 replication in myeloid cells[5,6].

The original papers describing SAMHD1 (refs. 5,6) assumed that this factor was not operating in T cells because the authors did not detect SAMHD1 expression in T cell lines. However, Baldauf *et al.*[3] found that supplying Vpx before or at the time of HIV-1 challenge greatly enhanced reverse transcription and HIV gene expression in resting T cells, as it had in myeloid cells. Consistent with these results, they showed that SAMHD1 is as highly expressed in resting primary human CD4+ T cells as in the THP-1 monocyte cell line, but is not expressed in Jurkat T cells (as previously reported). Vpx does not alter the quiescent state of resting T cells but elevates cellular dNTP concentrations to facilitate HIV reverse transcription by activating proteasomal degradation of SAMHD1. To confirm that SAMHD1 is the restriction factor overcome by Vpx, the authors knocked down SAMHD1 in resting CD4+ T cells and observed increased HIV replication[3]. Incubation of resting CD4+ T cells with dNTPs also enhanced their infection with HIV-1. The authors also found that resting CD4+ T cells from a patient with AGS with a nonsense mutation in *SAMHD1* are permissive for HIV-1. This result is consistent with a previous study that found that *SAMHD1* deficiency significantly enhanced HIV-1 replication in peripheral blood mononuclear cells (PBMCs) compared with normal controls, but only when the PBMCs were not activated[8].

Baldauf *et al.*[3] also made several unexpected observations about SAMHD1 expression. First, SAMHD1 expression was similar in resting and activated CD4+ T cells. As T cell activation is linked to cell proliferation and dividing cells need dNTPs for DNA replication, one would expect reduced SAMHD1 expression following T cell activation. This is not the case[3], suggesting that dNTP concentrations are maintained in activated T cells by other enzymes, that SAMHD1 activity is regulated post-translationally or requires interaction with other host factors, or that its expression is tightly controlled during the cell cycle and is only degraded transiently around the time of DNA synthesis when elevated dNTP concentrations are needed (only a fraction of activated T cells are in S phase at any time). Consistent with this last idea, the authors found that a proliferation marker (Ki67) and SAMHD1 were never concurrently expressed in immune cells in human tonsil sections[3]. However, a more careful analysis of SAMHD1 expression and dNTP concentrations (and HIV-1 susceptibility) in activated T cells at different phases of the cell cycle is also warranted.

Another unexpected finding by Baldauf *et al.*[3] was that SAMHD1 localizes to both the nucleus and cytoplasm in T cells and monocyte-derived macrophages, as previous studies suggested that SAMHD1 is mostly located in the nucleus[4,8–10]. Vpx arrives in the cytoplasm with the incoming virion, but it is actively transported to the nucleus. Where in the cell Vpx and SAMHD1 interact needs to be clarified, especially given that there are conflicting reports about whether leptomycin B, which inhibits nuclear export of proteins, interferes with Vpx-mediated SAMHD1 degradation[9,10].

But how much does SAMHD1 contribute to the overall restriction of HIV-1 replication in resting CD4+ T cells? Most of the experiments in this study[3] and in the previous myeloid cell papers[5–7,9–12] compared the effects of supplying Vpx or exogenous dNTPs or knocking down SAMHD1 on early steps in the viral life cycle by measuring the amounts of early reverse transcripts or HIV-1 long terminal repeat (LTR)-driven transcription from a single round of replication of an integrated reporter virus. Baldauf *et al.*[3] found that HIV-1 transcription from Vpx-treated resting CD4+

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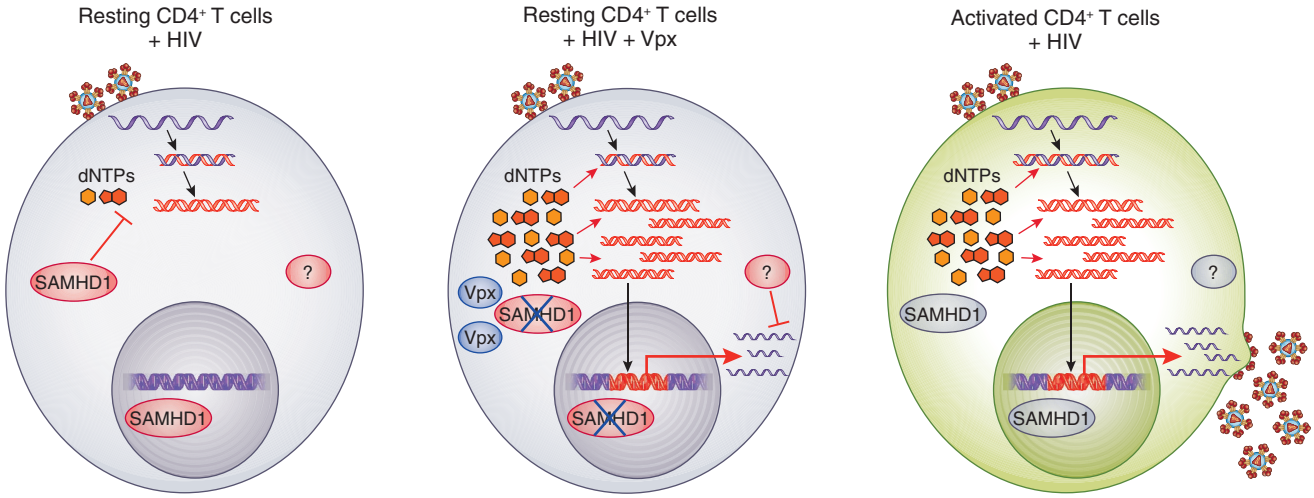

Figure 1 SAMHD1 restricts HIV-1 replication in resting CD4+ T cells by reducing dNTP concentrations needed for reverse transcription. Baldauf *et al.*[3] show that HIV infection of resting CD4+ T cells (left) is blocked by the SAMHD1 restriction factor early in the viral life cycle by limiting the amounts of dNTPs, which are needed to reverse transcribe genomic RNA (purple) into a cDNA copy (red). When they engineered HIV-1 virions to contain Vpx (normally present in HIV-2 but not HIV-1) (middle), they found that Vpx orchestrates the proteosomal degradation of SAMHD1, dNTP concentrations rise and reverse transcription, integration of the viral cDNA into the chromosome and transcription of HIV genes occurs. However, an unknown downstream block in these cells interferes with release of virions. In activated CD4+ T cells (right), despite high expression of SAMHD1, dNTP concentrations are elevated enough to support reverse transcription, and the viral life cycle goes to completion with release of infectious virus.

T cells is about 25% of that in Vpx-untreated activated CD4+ T cells from the same donor. The residual advantage to HIV-1 in activated T cells is probably explained by the greater nuclear abundance of transcription factors, such as NFAT, FOS, JUN and nuclear factor-κB, in activated T cells that are also required to turn on the HIV-1 LTR. However, viral reverse transcription, integration and transcription is not the whole story. The authors could not detect viral production in resting CD4+ T cells infected with Vpx-containing HIV-1; thus, unknown Vpx-insensitive blocks to HIV production occur at a later stage in the viral life cycle. It is unclear whether this might also be the case in myeloid cells. Although two studies have found that Vpx-treated monocyte-derived dendritic cells are more efficient at transmitting the virus to activated CD4+ T cells in *in vitro* co-culture[11,13], it is uncertain whether cell-free viruses were produced by the infected dendritic cells.

With this new study by Baldauf *et al.*[3], SAMHD1 is becoming an increasingly important host restriction factor for HIV-1 in both myeloid cells and resting CD4+ T cells. During HIV-1's evolution from primate lentiviruses, the accessory gene that counters SAMHD1 (*vpx*) was lost. Do loss of *vpx* and persistent SAMHD1 expression and the corresponding limit to infection in myeloid cells and resting CD4+ T cells provide an advantage to HIV-1? It would seem so. Although the simian immunodeficiency virus strain $SIV_{mac}239$, which lacks *vpx,* is slightly less pathogenic than the wild type SIV virus in rhesus monkeys[12], in humans, HIV-1 (which lacks *vpx*) is much more pathogenic than HIV-2 (which resembles macaque SIV and contains *vpx*). Although these findings are at first counterintuitive, the answer to why HIV-1 has lost *vpx* may lie in avoiding triggering an antiviral innate immune response in SAMHD1-restricted cells. When dendritic cells are infected with HIV-1 supplemented with Vpx, expression of interferon-β, a cytokine that orchestrates a global antiviral response, is induced[11,13]. It is not clear how Vpx-mediated degradation of SAMHD1 leads to interferon induction in myeloid cells. HIV-1 reverse transcripts also trigger interferon production in T cells and macrophages when another AGS-linked gene, *TREX1*, is knocked out (in mice) or inactivated (in human cells)[14]. Trex1 is a cytosolic DNase that digests excess nonproductive HIV reverse transcripts to avoid triggering interferon responses in activated CD4+ T cells and macrophages. Mutations in both *TREX1* and *SAMHD1* are linked to AGS, and both enzymes target HIV-1 reverse transcription: SAMHD1 controls DNA synthesis (via dNTP concentrations), whereas Trex1 controls degradation of reverse transcripts, much like a kitchen sink analogy in which SAMHD1 controls the faucet and Trex1 controls the drain. Therefore, HIV-1 may take advantage of both molecules to achieve an optimal viral cDNA concentration to promote viral transmission while evading innate immunity. Further studies are needed to understand whether and how HIV-1 restriction might enable HIV to avoid triggering antiviral innate immunity and how this might enhance viral transmission and infection.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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