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Low SAMHD1 expression following T-cell activation and proliferation renders CD4\(^+\) T cells susceptible to HIV-1

Nicolas Ruffin\(^{a,b,c}\), Vedran Brezar\(^{a,b,c}\), Diana Ayinde\(^{d}\), Cécile Lefebvre\(^{a,b,c}\), Julian Schulze Zur Wiesch\(^{e}\), Jan van Lunzen\(^{e}\), Maximilian Bockhorn\(^{f}\), Olivier Schwartz\(^{d}\), Hakim Hocini\(^{a,b,c}\), Jean-Daniel Lelievre\(^{a,b,c,g}\), Jacques Banchereau\(^{a,b,c,g,h}\), Yves Levy\(^{a,b,c,g}\) and Nabila Seddiki\(^{a,b,c}\)

**Objectives:** HIV-1 replication depends on the state of cell activation and division. It is established that SAMHD1 restricts HIV-1 infection of resting CD4\(^+\) T cells. The modulation of SAMHD1 expression during T-cell activation and proliferation, however, remains unclear, as well as a role for SAMHD1 during HIV-1 pathogenesis.

**Methods:** SAMHD1 expression was assessed in CD4\(^+\) T cells after their activation and in-vitro HIV-1 infection. We performed phenotype analyzes using flow cytometry on CD4\(^+\) T cells from peripheral blood and lymph nodes from cohorts of HIV-1-infected individuals under antiretroviral treatment or not, and controls.

**Results:** We show that SAMHD1 expression decreased during CD4\(^+\) T-cell proliferation in association with an increased susceptibility to in-vitro HIV-1 infection. Additionally, circulating memory CD4\(^+\) T cells are enriched in cells with low levels of SAMHD1. These SAMHD1\(^{\text{low}}\) cells are highly differentiated, exhibit a large proportion of Ki67\(^+\) cycling cells and are enriched in T-helper 17 cells. Importantly, memory SAMHD1\(^{\text{low}}\) cells were depleted from peripheral blood of HIV-infected individuals. We also found that follicular helper T cells present in secondary lymphoid organs lacked the expression of SAMHD1, which was accompanied by a higher susceptibility to HIV-1 infection in vitro.

**Conclusion:** We demonstrate that SAMHD1 expression is decreased during CD4\(^+\) T-cell activation and proliferation. Also, CD4\(^+\) T-cell subsets known to be more susceptible to HIV-1 infection, for example, T-helper 17 and follicular helper T cells, display lower levels of SAMHD1. These results pin point a role for SAMHD1 expression in HIV-1 infection and the concomitant depletion of CD4\(^+\) T cells.

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**Keywords:** HIV restriction, HIV-1, lymphocyte activation, SAMHD1, T-helper 17 cells
Introduction

The infection of CD4+ T cells by HIV-1 relies on the activation state of the cell [1,2]. It has long been known that activated cells are more prone to HIV-1 infection than quiescent CD4+ T cells [3]. SAMHD1 restricts productive HIV-1 infection in dendritic cells, myeloid cells [4,5] and resting CD4+ T cells [6,7]. Several mechanisms have been proposed for SAMHD1 blocking of HIV-1 infection. Indeed, SAMHD1 is a deoxynucleoside triphosphates (dNTPs) hydrolase and its expression decreases the intracellular dNTP pool thus blocking the reverse transcription [6–8]. Phosphorylation of SAMHD1, however, has been shown to inhibit the restriction activity of SAMHD1 [9–11], independently of the dNTPase capacity. The ribonuclease activity of SAMHD1 was recently reported to be crucial for HIV-1 restriction [12]. Of importance, several T-cell lines [4–6] as well as peripheral blood mononuclear cells (PBMCs) from patients suffering T-cell lymphoproliferative disease [13] display low levels of SAMHD1, illustrating the possible modulation of SAMHD1 expression.

Although the mechanisms of SAMHD1 restriction have been the subject of intense investigation, it remains to be determined whether SAMHD1 plays a role in HIV-1 pathogenesis. In the present article, we evaluated the regulation of SAMHD1 expression during CD4+ T-cell activation and found that SAMHD1 levels decrease with cell proliferation. Importantly, the activation-induced SAMHD1 decrease was associated with higher susceptibility to HIV-1 infection in vitro. We found that peripheral memory CD4+ T cells comprise a subset of cells expressing low levels of SAMHD1. Those CD4+ T cells are highly differentiated, enriched in cycling cells and are significantly decreased in HIV-infected individuals as compared with controls. In addition, peripheral T-helper 17 (Th17), as well as lymph nodes follicular helper T (Tfh) cells, two CD4+ T-cell subsets highly susceptible to HIV-1 infection, displayed the lowest expression of SAMHD1. Our results uncover that the regulation of SAMHD1 expression during T-cell activation and differentiation participate to the susceptibility of CD4+ T cells to HIV-1 infection.

Methods

Patient information

Ethical committee approval and written informed consent from all individuals or their parents, in accordance with the Declaration of Helsinki, were obtained prior to study initiation.

Peripheral blood was collected from healthy controls (n = 19) and HIV-1-infected individuals (n = 35) (Table, Supplemental Digital Content 1, http://links.lww.com/QAD/A646). Among the HIV-1-infected patients, 23 were receiving antiretroviral therapy (ART) [median (range) CD4+ T-cell count: 522 (129–1080) cells/µl; HIV-1 RNA: <20 (0–74042) copies/ml], six were either naive to treatment or under treatment interruption [median (range) CD4+ T-cell count: 255 (145–805) cells/µl; HIV-1 RNA: 54047 (982–158169) copies/ml], and six individuals were elite controllers [median (range) CD4+ T-cell count: 900 (737–1483) cells/µl; HIV-1 RNA: 94 (0–308) copies/ml]. Human peripheral blood and cord blood (n = 5) were obtained from Etablissement Français du Sang. PBMCs were isolated by Ficoll gradient centrifugation (PAAPartners, Velizy, France) and then either processed immediately or stored frozen until required for analysis.

Tonsils (n = 28) were obtained from children undergoing tonsillectomy at the Necker Hospital, Paris. Mechanical tissue disruption and 70 µm filtration were used to isolate single-cell suspension.

Surgically removed axillary lymph nodes from HIV-1-infected individuals (n = 6, CD4+ T-cell count: 318 (122–573) cells/µl; HIV-1 RNA <50 copies/ml) were obtained from the University Medical Center Eppendorf. HIV-negative lymph node samples (n = 6) were obtained from organ donors, who tested negative for HIV-1 and had no chronic infections, through National Disease Research Interchange, University Medical Center Eppendorf, and Columbia Center for Translational Immunology (Table, Supplemental Digital Content 2, http://links.lww.com/QAD/A646).

Ex-vivo T-cell phenotype

Multicolor flow cytometry was performed on fresh PBMCs and frozen LNMCs. T-cell subpopulations were determined using the following fluorochrome-conjugated antibodies: Pacific Blue, PE-Cy7 or Alexa-Fluor 700anti-CD3, PerCP or Alexa-Fluor 700 anti-CD4, PE anti-CD27, PE-Cy7 anti-CCR7, PE-CF594 anti-CD45R0, Alexa-Fluor 488 anti-CXCR5, Alexa-Fluor 647 anti-CXCR4, PE anti-CXCR3, PE-Cy7 anti-CCR6 (Becton Dickinson Biosciences, Pont de Claix (Le), France). Vioblue anti-CD3, APC-Vio770 anti-CD4, APC-Vio770 anti-CD45R0, APC anti-CD28 (Miltenyi Biotec, Paris, France). Brilliant Violet 421 anti-CD279 (Biolegend, London, UK).

Intracellular staining was carried out using the FoxP3 permeabilization solution kit according to the manufacturer’s instructions (eBioscience, Paris, France) together with PE Ki67, PE anti-Bcl-6 (BD Pharmingen) or PE anti-FoxP3 (Biolegend), and Alexa-Fluor 488 anti-SAMHD1 (a gift from O. Schwartz) or rabbit anti-SAMHD1 (Euromedex, Souffelweyersheim, France) followed by Alexa-Fluor 647 antirabbit antibody (Invitrogen, Life Technologies, Saint Auben, France).
In vitro T-cell proliferation

Purified CD4<sup>+</sup> T-lymphocytes from healthy individuals were separated from PBMCs using the CD4<sup>+</sup> T-cell Isolation Kit II (Miltenyi Biotech) and stained with either 0.25 μmol CFSE or 0.5 μmol Cell Trace Violet (Invitrogen). Purified CD4<sup>+</sup> T cells (1 × 10<sup>5</sup>) were cultured in RPMI-1640 (Life Technologies) containing t-glutamine, 10% fetal calf serum and antibiotics (penicillin and streptomycin) on a precoated 48-well plate with anti-CD3 (2 μg/ml) and anti-CD28 (2 μg/ml) (Beckman Coulter, Villepinte, France). After 72 h, cells were stained with Viobrite anti-CD3, APC-Vio770 anti-CD4 (Miltenyi Biotech) together with the Aqua Live/Death Vivid detection kit (Invitrogen). Samples were fixed and permeabilized using the FoxP3 permeabilization kit (eBioscience) and further stained with either Alexa Fluor 488 anti-SAMHD1 (a gift from O. Schwartz) or rabbit anti-SAMHD1 (Eurodix) followed by Alexa-Fluor 647 antirabbit antibody (Invitrogen). Fluorescence intensities were measured as described previously. Alternatively, CFSE-labeled cells were sorted according to CFSE fluorescence intensity using a MoFlo Legacy (Beckman Coulter) for mRNA quantification.

In-vitro HIV-1 infection

Purified CD4<sup>+</sup> T cells from PBMCs were separated using the CD4<sup>+</sup> T-cell Isolation Kit II (Miltenyi Biotech) and stained with Cell Trace Violet (Invitrogen). CD4<sup>+</sup> T cells (1 × 10<sup>5</sup>) were cultured in RPMI-1640 containing t-glutamine, 10% fetal calf serum and antibiotics (penicillin and streptomycin) on a precoated 48-well plate with anti-CD3 (2 μg/ml) and anti-CD28 (2 μg/ml) (Beckman Coulter). After 72 h, cells were incubated for 2 h in the presence of 100 ng NL4.3 HIV-1 lab strain virus. We used a wild-type HIV-1, NL4.3 strain, pseudotyped with Vesicular stomatitis virus G glycoprotein (VSV-G) produced in HEK 293T cells as described previously [14]. After washing in RPMI medium, cells were further cultured for 2–3 days on a precoated 48-well plate with anti-CD3 (2 μg/ml) and anti-CD28 (2 μg/ml). The reverse transcriptase inhibitor nevirapine (used at a final concentration of 25 nmol) was from the NIH AIDS Reagents Program. Cells were stained with aqua Vivid and fixed in PFA 4% in PBS for 15 min before a 20-min incubation in PBS containing 0.5% Triton X-100 (Sigma-Aldrich, St Quentin Fallavier, France) for permeabilization. Alexa-Fluor 488 anti-SAMHD1 and RD1 KC57 (antip24, Beckman Coulter) were used for intracellular staining as described above.

Results

TCR triggering induces the decreased expression of SAMHD1 in CD4<sup>+</sup> T cells

Resting CD4<sup>+</sup> T cells express SAMHD1, preventing their infection by HIV-1 [6,7]. The activation of CD4<sup>+</sup> T cells is thought not to modify the levels of SAMHD1 expression [6,10]. We used anti-CD3 and anti-CD28 antibodies to activate CD4<sup>+</sup> T cells and establish whether the expression of SAMHD1 can be modulated during T-cell proliferation. As shown in Fig. 1a, the levels of SAMHD1 gradually decreased with CD4<sup>+</sup> T-cell divisions to reach a plateau after four cycles of division. The decrease in protein expression was also associated with decreased SAMHD1-mRNA in proliferating-cells (Fig. 1b). These results are in contrast to previous publication using different activation [6] and/or measuring SAMHD1 expression on the bulk of CD4<sup>+</sup> T cells [6,10]. When using phytohemagglutinin and interleukin-2 (PHA/interleukin-2), proliferating CD4<sup>+</sup> T cells similarly decreased their expression of SAMHD1 (Figure, SuppDigital Content 3, http://links.lww.com/QAD/A646). We then confirmed that cells expressing lower levels of SAMHD1 were more susceptible to HIV-1 infection as described above.
infection in vitro. CD4+ T cells were activated for 72 h with anti-CD3/CD28 and then infected for 2 h with the HIV-1 NL4.3 strain. Despite similar levels of phosphorylated SAMHD1 (Figure, Supplemental Digital Content 4, http://links.lww.com/QAD/A646), proliferating cells showed the highest levels of intracellular p24 (Fig. 1c and d). Notably, there was an inverse correlation between the levels of SAMHD1 and the percentages of p24+ cells (P = 0.016; Fig. 1d and e). Thus, the activation of CD4+ T cells induces a downregulation of SAMHD1 expression in dividing CD4+ T cells that associates with an increased susceptibility to HIV-1 infection in vitro.
CD4⁺ T cells expressing low SAMHD1 are enriched in cycling cells and are depleted during HIV-1 infection

The hallmark of HIV-1 infection is the chronic immune activation [15]. To evaluate whether SAMHD1 expression was also modulated during HIV-1 infection, we assessed its expression in different subsets of CD4⁺ T cells. As shown previously [6,7], although SAMHD1 was absent in a proportion of lymphocytes (43 ± 13% of non-T cells), it was expressed by most CD3⁺ T cells (94 ± 7%) (Figure, Supplemental Digital Content 5, http://links.lww.com/QAD/A646). Among CD4⁺ T cells, we observed that a subset of memory cells displayed low levels of SAMHD1 (Fig. 2a). These SAMHD1low cells were preferentially in the memory cell compartment of adult PBMCs and could not be identified within cord blood (Fig. 2a). Importantly, ART-treated patients (n = 23), non-ART-treated patients (n = 6) and elite-controllers (n = 6) showed 4.14 ± 2.95, 5.84 ± 3.01 and 5.29 ± 3.3% of SAMHD1low cells in the blood CD4⁺ T-cell memory compartment, respectively, values significantly lower than those of HIV-negative controls (n = 13, 10.86 ± 5.83%) (data not shown). Deconvolution of the CD4⁺ T-cell compartment revealed lower proportions of SAMHD1low cells in transitional memory T EM (CCR7⁺CD45RO⁺/RA⁻), effector memory T EM (CCR7⁻CD45RO⁺/RA⁻) and terminally differentiated T TD (CCR7⁺CD27⁻CD45RO⁺/RA⁻) in HIV-positive as compared with HIV-negative individuals (Fig. 2b and c). In contrast, HIV-positive and HIV-negative individuals showed similar numbers of SAMHD1low cells within naive (CCR7⁺CD27⁺CD45RO⁺/RA⁻) and central-memory T CM (CCR7⁻CD27⁺CD45RO⁺/RA⁻) CD4⁺ T cells (Fig. 2e). Together these results reveal the presence of a highly differentiated SAMHD1low CD4⁺ T-cell subset in peripheral blood that is decreased in HIV-infected patients.

To evaluate whether SAMHD1low CD4⁺ T cells are activated in vivo, we measured Ki67 expression, as well as markers of T-cell activation on PBMCs from HIV-negative and HIV-positive individuals. As shown in Fig. 2d and e, SAMHD1low CD4⁺ T cells display high levels of Ki67 (P < 0.001 as compared with SAMHD1⁺ CD4⁺ T cells), thereby indicating their cycling status. SAMHD1low CD4⁺ T cells from non-treated HIV-infected individuals exhibited the highest proportion of Ki67⁺ cells (37.20 ± 27.56%) as compared with ART-treated individuals, elite controllers and healthy controls that showed 21.48 ± 19.71, 12.87 ± 5.49 and 12.23 ± 9.93%, respectively (P < 0.01). Gating strategies for representative patients in each group are shown in Supplemental Digital Content 6, http://links.lww.com/QAD/A646. In addition, SAMHD1low CD4⁺ T cells showed significantly higher levels of PD-1, CD38 and HLA-DR expression than SAMHD1⁺ CD4⁺ T cells (Figure, Supplemental Digital Content 7, http://links.lww.com/QAD/A646), confirming their activated state. Altogether, our data indicate that CD4⁺ T cells may be losing SAMHD1 expression during their differentiation and proliferation after antigen encounter in vivo.

The blood SAMHD1low CD4⁺ T-cell compartment decrease is restricted to T-helper 17 cells

As differential susceptibility to HIV-1 infection has been described for CD4⁺ T-cell subsets [16–19], we further examined the expression of SAMHD1 in Th1 (CD45RO⁺CXCR3⁺CCR4⁻CCR6⁺), Th2 (CD45RO⁺CXCR3⁻CCR4⁺CCR6⁺), Th17 (CD45RO⁺CXCR3⁻CCR4⁺CCR6⁻) [20], Tregs (CD45RO⁺CD25⁺CD127low) [21] and Tfh-like (CXCR5⁺PD1⁺) cells [22,23] (Fig. 3a). Proportions of SAMHD1low cells were comparable in HIV-negative and HIV-positive individuals in all subsets except Th17 (Fig. 3b and c). Indeed, 12.22 ± 3.01% of Th17 cells expressed low levels of SAMHD1 in HIV-negative controls as compared with 6.42 ± 4.39% in HIV+ cART⁺ (P < 0.001), 5.50 ± 4.30% in HIV+cART⁻ (P < 0.01) and 8.56 ± 2.20% in HIV⁻EC (P > 0.05) individuals (Fig. 3c). Moreover, Th17 cells were enriched in the SAMHD1low compartment compared with SAMHD1⁺, and there was a significant decrease of SAMHD1low cells among Th17 in HIV⁻ individuals [18.38 ± 6.57% in HIV⁻ vs. 10.66 ± 5.77% in HIV+cART⁺ (P < 0.001), 7.11 ± 6.62% in HIV⁺cART⁻ (P < 0.001) and 14.19 ± 3.41% in HIV⁻EC (P < 0.05); Fig. 3d]. The proportions of other Th subsets within SAMHD1low cells did not vary noticeably between our groups of HIV-negative and HIV-positive individuals, except for the Th2 population. Unlike for Th17 cells, however, we found lower proportions of Th2 cells in both SAMHD1low and SAMHD1⁺ compartments in HIV-infected individuals as compared with controls.

Consistent with their preferential depletion during HIV-1 infection [16–19], our results suggest that higher susceptibility of Th17 cells to HIV-1 infection may be linked to a lower expression of SAMHD1.

Follicular helper T cells from secondary lymphoid organs express low levels of SAMHD1 and are highly susceptible to HIV infection

Recent data indicated that CXCR5⁺ PD1⁺ Tfh cells in the lymph nodes were more susceptible to HIV-1 infection [24,25]. We investigated the presence of SAMHD1low CD4⁺ T cells in lymph nodes from both HIV-negative and HIV-positive cART-treated individuals (Fig. 4a). Flow cytometry analyses revealed that CXCR5⁺PD1⁺ Tfh cells were mostly SAMHD1low (Fig. 4a and b). The high levels of the transcription factor Bcl-6 in CXCR5⁺PD1⁺ CD4⁺ T cells from both groups further demonstrate that these cells are indeed Tfh cells (Fig. 4c). The frequency of CXCR5⁺PD1⁺ Tfh cells was...
**Fig. 2. Low SAMHD1 expression on differentiated and cycling CD4^+ T cells ex vivo.** Cord blood (n = 5) and peripheral blood mononuclear cells (PBMCs) from HIV-1 negative (HIV^−, n = 13) or HIV-1-infected individuals treated (HIV^+ cART^+, n = 23), nontreated (HIV^+ cART^−, n = 6) or elite controllers (HIV^+ EC, n = 6) were stained with anti-CD3, anti-CD4, anti-CD45RO or anti-CD45RA, anti-CCR7, anti-CD27, anti-CD28 and anti-SAMHD1. (a) Representative dot-plots of SAMHD1 and CD45RO expression in CD4^+ T cells from adult PBMCs and cord blood. (b) Gating strategy of CD4^+ T-cell subpopulations into naive (T_N: CD45RO^−CCR7^−CD27^+), central memory (T_CM: CD45RO^+CCR7^+CD27^+), transitional memory (T_TM: CD45RO^+CCR7^−CD27^+), effector memory (T_EM: CD45RO^+CCR7^−CD27^−) and terminally differentiated (T_TD: CD45RO^−CCR7^−CD27^−). (c) Percentages of SAMHD1^low^ cells among naive (T_N), central memory (T_CM), transitional memory (T_TM), and effector memory (T_EM), and terminally differentiated (T_TD) CD4^+ T cells. (d) Representative dot-plots of SAMHD1^+^ and SAMHD1^low^ cells from PBMCs isolated from healthy individuals (n = 13) and HIV-1-infected individuals treated (HIV^+ cART^+, n = 18), nontreated (HIV^+ cART^−, n = 5) or elites controllers (HIV^+ EC, n = 6). Data represent median with 25th–75th interquartile range with means displayed as ±. *P < 0.05, **P < 0.01, ***P < 0.001, two-way analysis of variance tests followed by Bonferroni posttests.
Fig. 3. SAMHD1<sub>low</sub> T-helper 17 cells are preferentially depleted during HIV-1 infection. Fresh peripheral blood mononuclear cells from HIV-1-negative (HIV-<sup>-</sup>, n = 13) or HIV-1-infected individuals treated (cART+ HIV<sup>+</sup>, n = 17), nontreated (cART– HIV<sup>+</sup>, n = 5) or elite controllers (HIV<sup>+</sup> EC, n = 6) were stained with anti-CD3, anti-CD4, anti-CD45RO, anti-CCR5, anti-CCR4, anti-CCR6 or anti-CD127 and anti-CD25 or anti-CXCR5 and anti-PD1, and anti-SAMHD1. Aqua Vivid was included in the procedure to remove dead cells from the analysis. (a) Gating strategy for memory T helper 1 (Th1: CD45RO<sup>+</sup> CXCR3<sup>+</sup> CCR4<sup>/</sup>C0<sup>/</sup> CCR6<sup>/</sup>C0<sup>/</sup> ), Th1/Th17 (CD45RO<sup>+</sup> CXCR3<sup>+</sup> CCR4<sup>/</sup>C0<sup>/</sup> CCR6<sup>+</sup> ), Th2 (CD45RO<sup>+</sup> CXCR3<sup>+</sup> CCR4<sup>/</sup>C0<sup>/</sup> CCR6<sup>/</sup>C0<sup>/</sup> ), Th17 (CD45RO<sup>+</sup> CXCR3<sup>/</sup>C0<sup>/</sup> CCR4<sup>+</sup> CCR6<sup>+</sup> ), Treg (CD45RO<sup>+</sup> CD127<sup>low</sup> CD25<sup>hi</sup> ) and Tfh-like (CD45RO<sup>+</sup> CXCR5<sup>+</sup> PD1<sup>+</sup> ) CD4<sup>+</sup> T cells. (b) Representative dot-plots of SAMHD1 expression within the Th17 subset gated as in (a) from an HIV-1 negative (HIV<sup>-</sup>), HIV-1 infected individuals treated (cART+ HIV<sup>+</sup>), nontreated (cART– HIV<sup>+</sup>) and elite-controllers (HIV<sup>+</sup> EC). (c) Percentages of SAMHD1<sub>low</sub> cells among Th17, Th1/17, Th1, Th2, Treg and Tfh-like CD4<sup>+</sup> T-cells. (d) Percentages of Th17, Th1/17, Th1, Th2, Treg and follicular helper T (Tfh) like cells among gated SAMHD1<sup>+</sup> and SAMHD1<sub>low</sub> CD4<sup>+</sup> T cells. Data represent median with 25th–75th interquartile range with means displayed as +. *P < 0.05, **P < 0.01 and ***P < 0.001, two-way analysis of variance tests followed by Bonferroni posttests.
not significantly different between treated-HIV-positive and HIV-negative individuals (data not shown), as described earlier [24,25]. Tfh from HIV-1-infected individuals produced higher levels of interleukin-21 and lower interleukin-17 as compared with HIV-negative individuals, whereas no difference in interferon-γ, tumor necrosis factor-α and interleukin-2 production was found (Figure, Supplemental Digital Content 8, http://links.lww.com/QAD/A646).

For further analysis, due to the scarcity of lymph nodes samples from healthy individuals, we used tonsils from HIV-negative individuals undergoing tonsillectomy. Tonsilar Tfh cells (CXCR5⁺PD1⁺PD1hiBcl-6), similar to Tfh from lymph nodes, expressed lower levels of SAMHD1 at both protein and mRNA levels when compared with CXCR5⁺PD1⁻, CXCR5⁺PD1int and CXCR5⁺PD1hi (Fig. 5a–c, and Figure, Supplemental Digital Content 9, http://links.lww.com/QAD/A646).

Fig. 4. Follicular helper T CD4⁺ T cells from lymph nodes express low levels of SAMHD1. LNMCs from HIV-negative (n = 6) and ART-treated HIV-1-infected individuals (n = 6) were stained for CD3, CD4, CXCR5, PD1, Bcl-6, SAMHD1 and Vivid. (a) Representative dotplots displaying CD45RO and SAMHD1 expression with the gating strategy for SAMHD1low cells on gated live CD4⁺ T lymphocytes. Left panel display the percentages of SAMHD1low cells among CD45RO⁺CD4⁺ T cells in HIV negative and treated-HIV-positive individuals. (b) Representative dot plots displaying PD1 and CXCR5 expression with gating strategy used. (c) Representative histogram showing Bcl-6 expression (left panel), and mean fluorescence intensity of Bcl-6 expression (right panel) in gated CXCR5⁻PD1⁻, CXCR5⁺PD1int, CXCR5⁺PD1⁺ and CXCR5⁺PD1hi CD4⁺ T-cell subpopulations. (d) Representative histogram showing SAMHD1 expression (left panel), and percentages of SAMHD1low cells (right panel) in gated CXCR5⁻PD1⁻, CXCR5⁺PD1int, CXCR5⁺PD1⁺ and CXCR5⁺PD1hi CD4⁺ T-cell subpopulations.
Moreover, CXCR5⁺PD1ʰ⁻Bcl-6⁺SAMHD1ₖₙ.low cells were Foxp3-negative, ruling out their regulatory Tfh phenotype and function [26]. They also expressed low levels of PRDM1/Blimp-1, the transcription factor that is mutually antagonistic with Bcl-6 [27]. CXCR5⁺PD1¹ⁿ cells expressed low levels of CD26, high levels of CD38, CD161, CD278 and CD152, and are enriched in Ki67⁺ cells (Figure, Supplemental Digital Content 10a, http://links.lww.com/QAD/A646), confirming their activated phenotype, in line with their function in the germinal center [28]. We have also found Tfh cells to express high and intermediate levels of CXCR4 and CCR5,

![Image](image-url)
Discussion

Although SAMHD1 restriction activity toward HIV-1 infection is well established in vitro [5,6,12], its importance in the course of HIV-1 pathogenesis remains largely unknown. We demonstrate here that SAMHD1 expression is regulated after T-cell activation and that SAMHD1low/CD4+ T cells are preferentially susceptible to HIV-1 infection. Our results enlighten a possible role of SAMHD1 in the CD4+ T-cell depletion occurring in HIV-1-infected individuals.

We show that during CD4+ T-cell division, there is a downregulation of SAMHD1 at both mRNA and protein levels. Importantly, the decreased SAMHD1 expression during CD4+ T-cell proliferation correlated with an increased HIV-1 infection in vitro. Therefore, whereas SAMHD1 phosphorylation is an important determinant for HIV-1 restriction [9–12], we show that the protein expression, downregulated after T-cell activation, lead also to an increased susceptibility of CD4+ T cells to HIV-1 infection.

The mechanisms of CD4+ T-cell depletion during HIV-1 pathogenesis are multiple and remain a field of constant scientific investigation. It has been shown that the best correlate for CD4+ T-cell decline in HIV-1-infected individuals is the level of immune activation [31–33]. As SAMHD1 expression is modulated by CD4+ T-cell activation in vitro, we wondered if similar mechanisms could take place in vivo. By measuring SAMHD1 expression in peripheral CD4+ T cells, we reveal that a subset of memory cells displays low levels of SAMHD1 when compared with the majority of CD4+ T cells. SAMHD1low/CD4+ T cells are enriched in differentiated effector CD4+ T cells, the primary targets of HIV-1 [34]. In line with this, we found SAMHD1low/CD4+ T cells to be depleted in HIV-1-infected individuals, as compared with healthy controls, a finding consistent with SAMHD1 being a restriction factor for HIV-1 infection. Of note, we did not observe any differential expression of SAMHD1 between our different groups of HIV-infected individuals, in contrast to a recent study showing an increased SAMHD1-mRNA expression in EC mononuclear cells [35]. Our results strongly suggest that SAMHD1low/CD4+ T cells are depleted in HIV-1-infected individuals and are not recovered after ART initiation.

Notably, we found that peripheral SAMHD1low/CD4+ T cells express higher levels of Ki67 ex vivo, indicating that they are enriched in cycling cells. Memory SAMHD1low/CD4+ T cells also displayed higher expression of the activation markers CD38, HLA-DR and PD-1 as compared with SAMHD1+ cells. These results confirm that T-cell activation in vivo can also induce SAMHD1 downregulation. Thus, we uncover a new mechanism that may account for the high susceptibility toward HIV-1 infection of rapidly proliferating effector/memory CD4+ T cells. It might also be of interest to understand the molecular determinants modulating SAMHD1 expression. In particular, as some transcriptional factors are important for HIV-1 replication [36,37], the study of their relation with SAMHD1 expression may be of importance.

It is known that memory CD4+ T cells, the main targets of HIV-1 [38], are heterogeneous in their susceptibility to infection. Among the various subsets of CD4+ T cells, Th17 cells are presumed to be the most susceptible to HIV-1 infection and are preferentially depleted in infected individuals [16–19,39]. We found that Th17 cells exhibit the lowest levels of SAMHD1 in HIV-negative individuals. In addition, SAMHD1low/CD4+ Th17 cells are preferentially decreased in HIV-infected individuals as compared with controls, whereas SAMHD1+ Th17 cells were not affected. Unlike for Th17 cells, we found lower proportions of Th2 cells in both SAMHD1low and SAMHD1+ compartments, in HIV-infected individuals as compared with controls. These results suggest that the low levels of Th2 cell are independent of SAMHD1 expression and are more likely the consequence of antiviral immune responses. Our observation that SAMHD1low/Th17 cells were depleted in the blood of HIV-infected individuals but preserved in elite controllers brings to light a potential mechanistic link between loss of Th17, lack of SAMHD1 and HIV-1 infection. These results are in line with recent studies showing a role for SAMHD1 in the permissiveness of CD4+ memory T cells with stem cell-like properties (Tscm) to HIV-1 infection [40,41].

Lymphoid tissues are an important site for HIV-1 replication, with Th cells exhibiting the highest levels of viral replication, and thus contributing to HIV persistence [24,25]. In nontreated HIV-1-infected
individuals, despite high levels of viral replication, Thfh cell numbers are increased and act as an important contributor to the HIV-1 reservoir in vivo [24,25]. We demonstrate here that lymph nodes CXCR5hiPD1hiBcl-6hi Thfh cells lack SAMHD1 expression. Similar low expression of SAMHD1 was found in tonsilar Thfh cells and was associated with higher susceptibility toward HIV-1 infection. Indeed, non-Thfh CD4hi T-cell subsets that displayed higher expression levels of SAMHD1 exhibited lower HIV-1 infection. Of note, the discrepancies between lymph nodes Thfh and peripheral Thfh-like cells corroborate the interconnection between SAMHD1 levels and T-cell activation. Indeed, Thfh cells expressing Bcl-6 are found in the germinal center, specialized anatomic compartment of lymphoid tissues, and support B cell differentiation into plasma cells and the generation of potent antibody responses [28]. Thfh-like cells found in periphery are thought to arise from the differentiation of lymphoid Thfh into memory-like cells [42]. The Thfh-like resting phenotype would thus coincide with a high expression of SAMHD1. As Thfh functions are impaired during HIV-1 infection [43], a better understanding of molecular determinants leading to SAMHD1 modulation in Thfh cells may provide important insights indispensable for the generation of an efficient therapeutic vaccine.

Our work provides evidence that the lack of SAMHD1 expression plays an important role in the susceptibility of differentiated memory CD4hi T-cell subsets to HIV-1 infection in vivo. The understanding of SAMHD1 expression and its modulation may open new avenues for HIV research toward an HIV cure.

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Conflicts of interest

There are no conflicts of interest.

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