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REVIEW

Cell Research (2013) 23:876-885.

www.nature.com/cr

Restrictions to HIV-1 replication in resting CD4$^+$ T lymphocytes

Xiaoyu Pan[1], Hanna-Mari Baldauf[2], Oliver T Keppler[2], Oliver T Fackler[1]

[1]*Department of Infectious Diseases, Virology, University Hospital Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany;* [2]*Institute of Medical Virology, University of Frankfurt, Paul Ehrlich Str. 40, 60596 Frankfurt, Germany*

CD4$^+$ T lymphocytes represent the main target cell population of human immunodeficiency virus (HIV). In an activated state, CD4$^+$ T cells residing in lymphoid organs are a major reservoir of ongoing HIV-1 replication in infected individuals. In contrast, resting CD4$^+$ T cells are highly resistant to productive HIV-1 infection, yet are massively depleted during disease progression and represent a substantial latent reservoir for the virus *in vivo*. Barriers preventing replication of HIV-1 in resting CD4$^+$ T cells include a rigid layer of cortical actin and, early after HIV-1 entry, a block that limits reverse transcription of incoming viral RNA genomes. Defining the molecular bases of these restrictions has remained one of the central open questions in HIV research. Recent advances unraveled mechanisms by which HIV-1 bypasses the entry block and established the host cell restriction factor SAMHD1, a deoxynucleoside triphosphate triphosphohydrolase, as a central determinant of the cellular restriction to HIV-1 reverse transcription in resting CD4$^+$ T cells. This review summarizes our current molecular and pathophysiological understanding of the multi-faceted interactions of HIV-1 with resting CD4$^+$ T lymphocytes.

Keywords**:** HIV; resting CD4 T cells; innate immunity; restriction factor

Cell Research (2013) **23**:876-885. doi:10.1038/cr.2013.74; published online 4 June 2013

Introduction

HIV/AIDS has become one of the most devastating pandemics in recorded history. The adverse socioeconomic impact of the HIV pandemic, particularly on Africa, is unprecedented. Despite recent advances in the treatment of HIV-infected individuals, HIV continues to surge unabatedly in developing countries and AIDS still persists as a major cause of morbidity in Western societies. The introduction of highly active antiretroviral therapy (HAART) has had a significant impact on HIV/AIDS in the developed world, and these drugs have prolonged survival times, reduced viral load, and alleviated much suffering. However, the incidence of side effects and viral drug resistance is high and the drugs are unaffordable and unavailable in many parts of the developing world. The era of highly effective viral suppression by HAART is an era of successful virus containment, but by no means an era of cure. HAART has provided us with the opportunity to refocus our attention on studies relating to the complex pathogenesis of the disease and on the development of strategies to eradicate HIV from the body of infected individuals. Both activated and resting CD4$^+$ T cells play a pivotal role in these scenarios.

As an obligate cell parasite, HIV like any other virus, relies strictly on the presence of suitable host cell machinery for most of the steps of its life cycle. These essential host co-factors are recruited and/or manipulated by the virus to optimize the generation of viral progeny. However, a distinct set of cellular factors have also evolved to counteract the replication of viral pathogens such as HIV. These so called restriction factors constitute physical or functional barriers of the host cell to virus replication by interfering with specific steps of the viral life cycle. Restriction factors are often expressed constitutively but can frequently be induced by host interferon responses. In addition to the classical cellular and humoral as well as signalling-based innate immune responses, restriction factors are also viewed as an intrinsic arm of

Correspondence: Oliver T Fackler[a], Oliver T Keppler[b]
[a]Tel: +49-(0)6221-561322; Fax: +49-(0)6221-565003
E-mail: oliver.fackler@med.uni-heidelberg.de
[b]Tel: +49-(0)69-6301-5219; Fax: +49-(0)69-6301-6477
E-mail: Oliver.Keppler@kgu.de

the host immune response (see [1-4] for general reviews on HIV restriction factors).

Reflecting the need for specific host cell molecules that facilitate viral entry into target cells, the tropism of HIV-1 is primarily governed by the expression of the entry receptor complex consisting of CD4 and CXCR4 or CCR5 on the surface of target cells [5]. This receptor complex specificity is the major determinant of the cell tropism of HIV-1. $CD4^+$ T lymphocytes and cells of the monocytes/macrophage lineage are the predominant target cells of the virus, along with additional, less abundant cell populations such as dendritic cells (DCs) that can also be infected with HIV-1. Macrophages and DCs constitute important reservoirs for HIV replication as well as persistence and contribute to various aspects of disease progression. The differentiation state of these myeloid cells is an important determinant of their overall permissivity to HIV infection and/or response pattern to virus exposure [6, 7].

In the case of primary human $CD4^+$ T lymphocytes, the permissivity to HIV-1 infection *ex vivo* depends more on the cellular activation state: while activated, proliferating $CD4^+$ T lymphocytes are highly susceptible to infection and support efficient virus replication (Figure 1A), resting $CD4^+$ T cells are largely non-permissive for HIV-1 replication but can be infected *in vivo* and serve as a latent viral reservoir (Figure 1B) [8]. Since physiologically the vast majority of all $CD4^+$ T lymphocytes are in a resting state, this phenomenon provides one explanation as to why the frequency of productively infected $CD4^+$ T lymphocytes in AIDS patients is very low [9].

The resistance of resting $CD4^+$ T lymphocytes to HIV-1 replication *ex vivo* has been noted already in the early days of HIV-1 research where experiments demonstrated the requirement of T cell activation for full support of all steps of the viral life cycle [10-12]. Soon thereafter, it became clear that HIV-1 readily enters these target cells; however, the virus fails to complete the reverse transcription (RT) of incoming viral RNA genomes into DNA and thus fails to integrate its genome into that of the host cell [11, 13]. While extrachromosomal, frequently linear copies of the viral genome persist for extended periods of time in the nucleus of these cells, which could even allow for some viral gene expression [14], this does not permit the generation of viral progeny and virus spread. These initial studies pinpointed RT as a critical limitation step for HIV-1 replication in resting $CD4^+$ T lymphocytes and this barrier has been subsequently confirmed as the most potent post-entry hurdle to HIV-1 infection in resting $CD4^+$ T lymphocytes (Figure 1B) [15-19]. Depending on the precise experimental conditions used, however, multiple additional blocks were also described that limit HIV-1 replication at different steps of the viral life cycle. A physical barrier for incoming virions is imposed by cortical actin that can be surmounted by envelope glycoprotein-induced signaling via CXCR4 [20]. Additional blocks are less well understood and not overcome by intrinsic strategies of HIV-1, including suppression of transcriptional activity of integrated proviral copies by the host cell factor Murr1 [16], and a molecularly undefined block to virus release [21]. This review summarizes our current understanding of how $CD4^+$ T lymphocytes impose these individual barriers, describes mechanisms by which HIV-1 seeks to overcome or exploit these blocks, and discusses potential pathophysiological consequences of productive and non-productive HIV-1 replication in this large reservoir of target cells.

Cortical actin as a barrier for HIV-1 entry into resting $CD4^+$ T lymphocytes

Although fusion of incoming HIV-1 virions may be somewhat less efficient in resting naïve than in resting memory cells [22], HIV-1 particles efficiently enter resting $CD4^+$ T lymphocytes. Thus, there is apparently no general barrier that prevents virus interactions with the entry receptor complex and delivery of the viral genome to the target cell cytoplasm. Efficient execution of these steps, however, was suggested to depend on a signalling cascade that is triggered upon engagement of the chemokine receptor and entry co-receptor CXCR4 by the viral glycoprotein Env [20]. One downstream target of the elicited signalling cascade is the actin severing factor cofilin that is activated during virus entry (reviewed in [23, 24]). These results revealed that the cortical actin network, in principle, imposes a critical physical barrier for HIV-1 entry, which is however not apparent in the context of infection with X4-tropic HIV-1 due to the triggering of cofilin-activating signals. Cortical actin is more dynamic in T lymphocyte populations with higher permissivity to HIV-1 infection, suggesting that HIV-1 has to overcome this block specifically for entry into resting $CD4^+$ T lymphocytes [25] (Figure 1A and 1B). Importantly, the Env-CXCR4 interaction is strictly required and cannot be bypassed by replacing HIV-1 Env with the glycoprotein of the vesicular stomatitis virus (VSV-G): VSV-G pseudotyping funnels virus uptake to an endocytic route that apparently results in particle degradation within 2 days. While VSV-G-mediated HIV-1 entry is, therefore, largely non-productive in primary resting $CD4^+$ T-cells [22, 26, 27], transformed T-cell and fibroblast lines can readily be productively infected by HIV-1 pseudotyped with VSV-G. This also suggests that, in resting $CD4^+$ T lymphocytes, the CXCR4 signaling

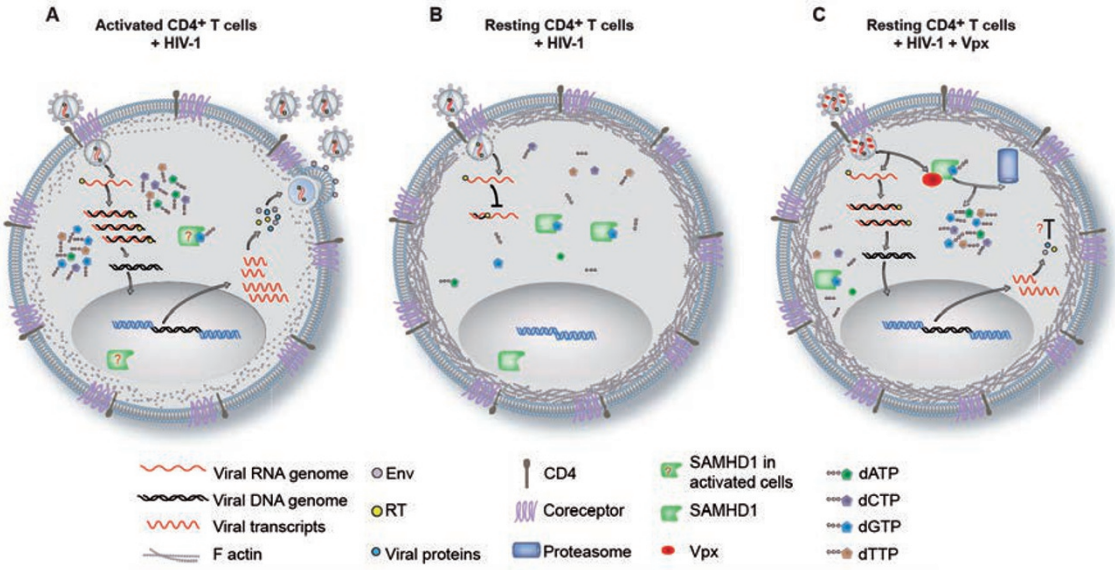

Figure 1 Schematic model of HIV-1 replication and restrictions thereof in activated and resting CD4+ T lymphocytes. **(A)** Activated CD4+ T lymphocytes are fully permissive to HIV-1 replication and all steps of the viral life cycle from entry (facilitated by loosening of the actin cortex), RT, nuclear import, integration, viral gene expression to synthesis of new viral proteins, particle assembly and release are efficient. Cellular dNTP levels are high despite the presence of high SAMHD1 expression, suggesting that the activity of the enzyme may be downregulated in these cells (indicated by the question mark). **(B)** HIV-1 infection of resting CD4+ T lymphocytes is abortive. HIV-1 overcomes the rigid actin cortex barrier by inducing Env-chemokine receptor signaling. RT is initiated but stalls before viral DNA synthesis is completed. Presumably due to the high activity of SAMHD1, dNTP levels in the cytoplasm are low and limit RT reactions. **(C)** Delivery of Vpx surmounts the SAMHD1 barrier and allows HIV-1 to infect resting CD4+ T lymphocytes. SAMHD1 is targeted by Vpx for proteasomal degradation, which is paralleled by an elevation of cellular dNTP levels. HIV-1 infection proceeds through RT, nuclear import and integration. Some viral gene expression is observed but no infectious progeny is released, suggesting the existence of yet uncharacterized additional blocks in the late phase of the viral life cycle.

induced by HIV-1 Env may indirectly facilitate steps in the early phase of the viral life cycle including RT, integration and viral gene expression. While receptor interactions by CCR5 (R5)-tropic HIV-1 are also known to elicit downstream signalling, it remains a matter of debate to which extent the resulting signals may also serve to overcome the cortical actin barrier to HIV-1 entry in resting CD4+ T lymphocytes (reviewed in [28]).

Impaired RT defines an early block to HIV-1 replication in resting CD4+ T lymphocytes

While several limiting steps for HIV to proceed through the replication cycle have been reported [11, 13, 16-18, 22], most studies highlighted an early post-entry block at the level of RT of the incoming HIV-1 RNA genome. As early as 1990, independent studies by Zack/Chen and Stevenson were the first to demonstrate that HIV-1 DNA synthesis is initiated in infected quiescent CD4+ T cells at levels comparable with those of activated T cells, but that the viral genome is not completely reverse-transcribed in these non-activated cells [11, 13]. Subsequent studies reported that exogenous addition of deoxynucleosides to cultured resting CD4+ T cells bypassed this block, allowing HIV to integrate in these cells [19, 29]. This indicated that nucleotide metabolism directly or indirectly affected the susceptibility of these primary target cells. The host cell restriction factor APOBEC3G was suggested by the Greene laboratory to play a critical role in limiting the permissivity of resting CD4+ T cells to HIV-1 infection, but this finding could not be subsequently confirmed by others [30, 31]. It thus remained unclear whether this block to HIV-1 RT in these cells reflects the lack of an essential host cell cofactor or the presence of an inhibitory factor.

Restriction of HIV-1 RT by SAMHD1 in resting CD4+ T lymphocytes

Insight into the molecular basis of the block to HIV-1 RT in resting CD4+ T lymphocytes was provided only recently by the identification of the deoxynucleoside tri-

phosphate (dNTP) triphosphohydrolase SAMHD1 (sterile alpha motif (SAM) and histidine/aspartic acid (HD) domain-containing protein 1) as an essential host cell factor for this restriction [32, 33] (see [34] for review). Previously, SAMHD1 had been shown to be expressed in cells of the myeloid lineage such as DCs, monocytes, and macrophages, and demonstrated to act as an infection barrier for HIV-1 that manifests its function at an early RT step [35-37]. This block to HIV-1 infection in myeloid cells resembled that observed in resting $CD4^+$ T lymphoyctes, yet the reported lack of SAMHD1 expression in T cell lines [36] appeared to exclude a role for the enzyme as an anti-HIV restriction factor in lymphoid cells; and thus the action of SAMHD1 was thought to be restricted to the myeloid lineage. Analysis of mRNA and protein expression in primary $CD4^+$ T lymphocytes, however, revealed abundant SAMHD1 expression [32, 33]. Importantly, resting $CD4^+$ T cells, in which SAMHD1 expression was (i) experimentally silenced by knockdown strategies or (ii) intrinsically lacking due to nonsense mutations in the *samhd1* gene, supported the early phase of HIV-1 infection including the completion of RT and early gene expression [32, 33]. Moreover, the accessory viral protein Vpx, which is encoded by HIV-2 and SIV variants, but not by HIV-1, and which is known to target SAMHD1 for proteasomal degradation in myeloid cells [35-37], also depleted SAMHD1 in $CD4^+$ T lymphocytes and facilitated HIV-1 RT [32, 33]. As reduction of SAMHD1 at the level of gene expression (gene silencing) or protein stability (Vpx) had no significant effect on the activation state of $CD4^+$ T lymphocytes, these findings establish SAMHD1 as a key restriction factor that inhibits HIV-1 replication in resting $CD4^+$ T lymphocytes at the level of RT.

Both studies that recently reported the role of SAMHD1 as an HIV-1 restriction factor in resting $CD4^+$ T lymphocytes agree on the overall relevance of this cellular enzyme in limiting the efficiency of HIV-1 RT [32, 33]. However, divergent results were obtained regarding the ability of HIV to express viral genes subsequent to overcoming the SAMHD1 barrier. One study showed that infection with replication competent, X4-tropic HIV-1 with virion-packaged Vpx resulted in the expression of a GFP reporter driven by the viral LTR promoter [32]. In contrast, infection with VSV-G-pseudotyped HIV-1 following the pre-loading of cells with Vpx-VLPs only resulted in GFP gene expression when the reporter gene was under the control of a CMV promoter, while the LTR-driven expression could not be detected [33]. The observation that anti-CXCR4 antibodies render resting T lymphocytes permissive for entry and completion of RT by VSV-G-pseudotyped HIV-1 [38] suggests that VSV-G does not elicit the necessary signaling pathways triggered by interactions of X4-tropic HIV-1 Env with the entry receptor complex (see above). Consequently, VSV-G pseudotypes (i) are much less efficient in mediating virus entry and subsequent SAMHD1 depletion in resting primary human $CD4^+$ T lymphocytes [27, 33] and (ii) do not provide cues for efficient transcription in phenotypically resting cells (also see [34]).

Mechanism of the SAMHD1 block to HIV-1 RT

As a dNTP triphosphohydrolase, SAMHD1 hydrolyzes dNTPs to deoxynucleosides in a dGTP-regulated manner [39]. Interestingly, cellular dNTP pools in monocyte-derived macrophages are approximately 100-fold lower compared with activated $CD4^+$ T cells [40]. For macrophages and DCs, a close functional and temporal correlation has been reported between the ability of Vpx to degrade SAMHD1, the elevation of cellular dNTP pools and the completion of RT of incoming HIV-1 [41, 42]. dNTP levels in resting $CD4^+$ T cells are also 10- to 100-fold lower than in their activated counterparts [32, 43]. These differences cannot be readily explained by differential expression of SAMHD1, as its overall expression levels and localization are comparable among all three conditions, suggesting that SAMHD1's function is likely regulated at the enzymatic activity level, e.g., through multimerization or posttranslational modifications. In this scenario, SAMHD1 hydrolyzes intracellular dNTP pools in non-cycling cells, decreasing their concentrations below a threshold required for efficient HIV-1 cDNA synthesis by the viral reverse transcriptase. Indeed, Vpx-mediated depletion of SAMHD1 in resting $CD4^+$ T lymphocytes was paralleled by an increase in cellular dNTP pools, and Vpx had no or little effect on HIV-1 infection in activated $CD4^+$ T lymphocytes with high dNTP levels [32]. These correlations are consistent with a direct link between Vpx-induced depletion of SAMHD1, alterations of the cellular dNTP pool and the permissivity of resting $CD4^+$ T lymphocytes to HIV-1 infection. However, the functional interconnection of these parameters still needs to be formally demonstrated and additional or alternative mechanisms can not be currently excluded.

Potential implications of the SAMHD1-mediated restriction

The HIV-1 pandemic is the result of multiple independent zoonotic events of simian immunodeficiency virus (SIV) transmission from monkeys to humans [44]. Antagonism of SAMHD1 restriction has been observed for Vpx as well as some ancestral Vpr vari-

ants of SIV and HIV-2, but HIV-1 does not encode a SAMHD1 antagonist [45, 46]. It is particularly intriguing that pandemic HIV-1 does not encode a functional SAMHD1 antagonist. As SAMHD1-antagonizing SIV is rarely pathogenic in its natural monkey host, the lack of SAMHD1 antagonism may have contributed to the pronounced pathogenic potential of HIV-1 in humans. This would predict that avoiding cells with potent SAMHD1 restriction in place elevates virulence and disease progression. One possible scenario could be that surmounting the SAMHD1 barrier would expose HIV-1 to potent innate immune recognition mechanisms that facilitate host control of the infection. Indeed, when bypassing the SAMHD1 barrier in monocytic cells and in DCs, HIV-1 infection elicits proinflammatory responses [37, 47]. Based on our current knowledge, the SAMHD1 block to early HIV-1 infection appears to be more pronounced in resting $CD4^+$ T lymphocytes than in most myeloid cells in which basal levels of infection can be achieved even in the context of SAMHD1 expression [37, 41]. It will, therefore, be of great importance to determine the breadth and magnitude of innate immune responses to HIV-1 infection of resting $CD4^+$ T lymphocytes.

ssDNA by-products of HIV-1 RT reactions can be recognized by so far unknown cytoplasmic sensor molecules and thereby trigger type I interferon responses [15, 48, 49]. However, this sensing mechanism can in principle be prevented in HIV-1-infected cells by the activity of the cellular exonuclease TREX1 that dampens innate immune recognition of HIV-1 infection by eliminating the accumulating viral ssDNA products [50]. This suggests that from the point of view of HIV, SAMHD1 and TREX1 "synergize" to protect the virus from innate immune recognition.

Of clinical importance, mutations within the *SAMHD1* and *TREX1* genes lead to a rare genetic disorder, the Aicardi-Goutières syndrome (AGS), the sympton of which resemble an immune system's response pattern to chronic viral infections, with a massive production of the antiviral cytokine interferon alpha (IFNα) and a detrimental activation of the immune system (see [51, 52] for reviews). These deficiencies result in a complex disease characterized by hepatosplenomegaly, chronic cerebrospinal fluid lymphocytosis, and an early-onset encephalopathy that leads to severe intellectual and physical handicap. As proposed for the case of HIV infection (see above), the common clinical manifestations of SAMHD1 and TREX1 deficiencies in AGS patients argue for synergistic and common effects of both enzymes in controlling nucleotide metabolism and innate immune responses.

If SAMHD1 and TREX1 indeed act to prevent an active and certainly beneficial immune response against a major human pathogen, why are they evolutionarily maintained? The answer may lie in the abundant presence of transcriptionally active endogenous retroelements in the human genome. Nucleic acids derived from these elements represent a constant source of molecular patterns, the sensing of which could have deleterious consequences. TREX1 thus likely has emerged to eliminate these triggers of proinflammatory responses and to avoid autoimmunity against retrolements [53, 54]. As a very recent addition to the list of human pathogens, HIV-1 thus has taken advantage of the human adaptation to its endogenous ancestors. In this scenario, SAMHD1 would simply act as a first barrier that significantly reduces the amount of exogenously delivered HIV-1 RT by-products, which are then eliminated by TREX1.

Recent work using human tonsil aggregate cultures, in which $CD4^+$ T lymphocytes with a resting phenotype can be infected with HIV-1 without exogenous stimulation [55, 56], however indicates that this evasion of sensing may not be complete and that the residual immune recognition may be of pathophysiological relevance. These infected cultures mirror a hallmark of AIDS pathogenesis in that seemingly uninfected bystander $CD4^+$ T lymphocytes are killed as a consequence of HIV-1 infection, leading to massive depletion of $CD4^+$ T lymphocytes [57, 58]. This effect was demonstrated to result from abortive infection of target $CD4^+$ T lymphocytes in which infection up to the level of an initiated RT was sensed and a proinflammatory response generated, ultimately leading to death of the infected resting $CD4^+$ T cells [15]. These findings imply that under the conditions used in this cell model, the SAMHD1/TREX1 barrier may not be fully functional. It will thus be of major interest to decipher the regulation of SAMHD1's and TREX1's activities in preventing the sensing of HIV-1 infection as well as the detailed mechanisms and consequences of this sensing process.

A block to HIV-1 transcription in resting $CD4^+$ T lymphocytes

Reflecting the lack of exogenous stimuli that trigger T-cell receptor signalling and cell proliferation, many transcriptional programs are dormant in resting $CD4^+$ T lymphocytes. Transcription of HIV-1 genes is subject to complex control and regulation by the viral transactivator Tat that ensures elongation of viral transcripts initiated by RNA polymerase II via association with the pTEFb complex (see review [59]). Expression and activity levels of the essential pTEFb components CyclinT1 and CDK9 are lower in resting than in activated T lymphocytes [60-62]. However, significant levels of Tat transactivation

of viral gene expression were observed in resting $CD4^+$ T lymphocytes [63, 64], suggesting that Tat activity *per se* may not be the key limitation to HIV-1 transcription in these target cells. The block to HIV-1 transcription in resting $CD4^+$ T lymphocytes may thus rather reflect limitations in transcriptional initiation, which relies on host cell transcription factors such as NF-κB, NF-AT and SP-1, that all recognize specific target sites in the viral promoter, the long terminal repeats (LTRs) (see review [65]). This cellular transcriptional control is instrumental for the regulation of latency, and also determines the ability of HIV-1 to efficiently transcribe viral genes and thus replicate in $CD4^+$ T lymphocytes [66-68]. As NF-κB and NF-AT are the central transcription factors triggered by T-cell activation [69], it is not surprising that their activity is limiting for HIV-1 transcription initiation and thus replication in resting $CD4^+$ T lymphocytes. This limited availability of cellular transcription factors results from a lack of stimulation but may also reflect the presence of regulatory mechanisms that keep levels of active NF-κB to a minimum in these cells: The Murr1 protein, also referred to as COMMD1, was reported to prevent proteasomal degradation of the negative regulator of NF-κB, IκB, thereby maintaining NF-κB in an inactive state and suppressing the transcription of HIV-1 genes [16]. While the specific role of Murr1/COMMD1 in this process still awaits independent confirmation, experimental interference with nucleo-cytoplasmic shuttling of IκB led to a boost in HIV-1 transcription when IκB was excluded from the nucleus [70]. Similarly, transcription of viral genes can be triggered by a splice variant of the transcription factor Ets-1 without requiring full cell activation [27]. Given the presence of these barriers to the initiation of HIV-1 transcription in the host cell, it is somewhat surprising that overcoming the SAMHD1 block to HIV-1 RT is sufficient to allow for the detection of early viral gene expression without triggering overt T-cell activation [32]. As viral gene expression in resting $CD4^+$ T cells was not observed using VSV-G-pseudotyped HIV-1 particles [33], it seems likely that HIV-1 transcription is facilitated by Env-CXCR4-mediated signalling that elevates the activity of HIV-1 transcription initiation factors to support HIV gene expression.

Block to the late phase of HIV-1 replication in resting $CD4^+$ T lymphocytes?

Overcoming the SAMHD1 barrier to HIV-1 RT and induction of HIV-1 gene expression by Vpx-mediated depletion of the restriction factor still did not allow the infecting virus to complete its replication cycle and release new viral progeny into the cell culture supernatant [32]. This result is consistent with earlier work showing that surmounting the block to HIV-1 RT by addition of deoxynucleosides (dN) also did not lead to completion of the viral life cycle [19, 29]. This indicates that blocks other than that mediated by SAMHD1 and that are not overcome by Vpx or experimental elevation of the cytoplasmic dNTP pool inhibit steps in the late phase of the viral life cycle somewhere between early gene expression and virus budding (Figure 1C). Such a late-phase block to HIV-1 replication has already been described in resting T cells that were rendered permissive to HIV-1 infection by co-culture with B lymphocytes and infected macrophages [21]. Owing to the activity of the viral protein Nef, macrophage infection resulted in the release of soluble CD23 and ICAM molecules, which triggered up-regulation of co-stimulatory receptors on B lymphocytes. These co-stimulatory receptors in turn rendered resting $CD4^+$ T lymphocytes susceptible to the early steps of HIV-1 infection including RT and viral gene expression, but without inducing T cell activation markers or cell proliferation. Of note, release of viral progeny and virus spread could not be observed under these conditions, illustrating the existence of a yet uncharacterized block in the late phase of the viral life cycle. The permissivity state of $CD4^+$ T lymphocytes induced by this paracrine mechanism shares striking similarities to the scenario observed in resting $CD4^+$ T lymphocytes upon dN treatment or infection with Vpx-containing HIV-1 [32]. This mechanism might also explain why in *ex vivo* cultures of human tonsils, in which macrophages and B lymphocytes are present, phenotypically resting $CD4^+$ T lymphocytes are permissive to HIV-1 infection [55]. It will be of great interest to analyze whether and by which mechanism this paracrine sensitization overcomes the SAMHD1 block. Equally relevant will it be to define the nature of the late phase block in resting $CD4^+$ T lymphocytes.

The study by Swingler *et al*. using the paracrine feedback system or the report by Baldauf *et al*. exploiting virion incorporation of Vpx, detected viral-gene expression via reporter genes expressed from the HIV-1 genome and did not provide a full assessment of viral transcripts and proteins synthesized under these conditions [21, 32]. It thus remains possible that steps from splicing, nuclear RNA export [71, 72], protein synthesis to assembly and egress are affected to some extent. In principle, components of host-cell machineries, such as the ESCRT complex, required for HIV-1 particle release may be subject to regulation at the level of expression or activity in T lymphocytes in an activation-dependent manner. On the other hand, multiple HIV-1 restriction factors inhibiting specific steps in the late phase of HIV-1 replication have already been described. The list includes the recently

identified HIV-1 restriction factor Schlafen 11 that interferes with late steps of the HIV-1 life cycle by reducing the expression of viral proteins in a codon-usage-dependent manner [73, 74]. Acting more directly on the release step, 2′,3′-cyclic-nucleotide 3′-phosphodiesterase reduces the assembly and budding of infectious HIV-1 in some cell types [75]. Another potent barrier to HIV-1 release is mounted by CD317/tetherin that tethers budding virions to the cell surface to prevent their efficient release and senses assembling viral capsids to trigger proinflammatory signalling [76-79]. Both these activities of CD317/tetherin are antagonized by the viral protein Vpu, but whether these restrictions can be counteracted by Vpu in resting $CD4^+$ T lymphocytes is unclear. Finally, several members of the TRIM E3 ubiquitin ligase family are known to interfere with HIV-1 particle release by still ill-defined mechanisms [80]. As expression and potency of these antiviral host cell factors have not yet been studied in resting $CD4^+$ T lymphocytes, characterizing their contribution to the late-phase block to HIV-1 replication in this cell type represents a major task of future studies.

Resting $CD4^+$ T lymphocytes as a latent reservoir

AIDS patients bear an important reservoir of latently HIV-1-infected resting $CD4^+$ T lymphocytes, from which virus replication can be initiated by cell activation such as antigenic stimulation. Because of its persistence during effective antiretroviral therapy and in spite of the low frequency of such latently infected resting $CD4^+$ T lymphocytes, this reservoir represents one of the main barriers towards eradication of HIV-1 in infected individuals. Latency is predominately determined at the level of transcription, leading to intense efforts to therapeutically activate transcription of the latent provirus towards the goal of eradication of the infection [81]. But, how can such a latent reservoir be established in light of the intrinsic resistance of resting $CD4^+$ T lymphocytes to the early phase of HIV-1 infection? One explanation may be provided by the complex interplay between immune cells in lymphoid tissues that, via paracrine or other mechanisms [21, 55], surmounts the barriers to the early phase of infection without activating target $CD4^+$ T lymphocytes. This scenario could account for the populations of infected resting naïve $CD4^+$ T lymphocytes observed *in vivo* [82-84]. In contrast, HIV-1-infected resting memory $CD4^+$ T lymphocytes that are more frequently detected in target organs *in vivo*, are more likely to mirror a post-activation state, in which infection and subsequent return to a lower activation level have occurred. It will be of great interest to analyze whether and how individual mechanisms to control HIV-1 replication differ in resting $CD4^+$ T lymphocytes during acute and latent HIV-1 infection.

Conclusions and perspectives

With SAMHD1, a key molecular player was recently identified that determines the resistance of resting $CD4^+$ T lymphocytes to HIV-1 replication. Important mechanistic questions that need to be addressed in this field include the regulation of SAMHD1 activity in the context of cell activation and complex lymphatic environments, the relationship between SAMHD1 depletion, alterations of nucleotide pools and permissivity to infection, as well

Glossary

AGS	Aicardi-Goutières syndrome, rare inherited autoimmune, inflammatory disorder caused by non-sense mutations in the *samhd1, trex1* or *rnaseh2a-c* genes
Cofilin	actin severing factor regulating cellular F-actin polymerization states
Cortical actin	F-actin layer underneath the plasma membrane providing a physical barrier to virus entry
CXCR4	chemokine receptor acting as entry co-receptor for HIV-1 with CXCR4-tropic Env
HIV	human immunodeficiency virus, causative agent of AIDS
RT	reverse transcription, characteristic step in the life cycle of retroviruses in which incoming genomic RNA is transcribed into DNA by the viral enzyme reverse transcriptase
SAMHD1	sterile alpha motif (SAM) and histidine/aspartic acid (HD) domain-containing protein 1, host cell deoxynucleoside triphosphate (dNTP) triphosphohydrolase
SIV	simian immunodeficiency virus
TREX1	three prime repair exonuclease 1, 3′-5′ cellular exonuclease
Vpx	viral protein X, encoded by most SIVs and HIV-2 strains, SAMHD1 antagonist
Vpr	viral protein R, encoded by HIV-1 and some SIV strains
VSV-G	glycoprotein of the vesicular stomatitis virus; can be incorporated into lentiviral particles to mediate cell entry in a CD4- and CXCR4-independent manner

as the role of SAMHD1 in infections by pathogens other than HIV-1. Furthermore, surmounting the SAMHD1 barrier by Vpx-mediated degradation unveiled additional blocks to HIV-1 spread in this abundant target cell population that now await further characterization. Further experimental investigations will open new avenues for deciphering the pathophysiological consequences of HIV-1 infection of resting $CD4^+$ T lymphocytes. Finally, findings deduced from such studies will likely prove relevant in the context of viral latency, and thus in the eradication of the virus.

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