The SAMHD1 knockout mouse model: *in vivo* veritas?

Ferdinand Roesch and Olivier Schwartz*

Institut Pasteur, Department of Virology; CNRS; and Université Paris Diderot, Sorbonne Paris Cité, Paris, France.

*Correspondence to: olivier.schwartz@pasteur.fr

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SAMHD1, a dNTP hydrolase mutated in the autoimmune encephalopathy Aicardi-Goutières syndrome, restricts HIV replication in non-dividing human cells by reducing intracellular deoxyribonucleotide pools. New work in *The EMBO Journal* unexpectedly finds neither autoimmune disease nor increased murine retrovirus infection in SAMHD1 knockout mice, but improved replication of a mutant HIV with increased sensitivity to low dNTP levels. Thus, while the new mouse model partially recapitulates known features of human SAMHD1, it represents a unique tool to study the role of dNTP regulation during inflammation, viral infection and other pathologies.

Upon entry into host cells, viruses encounter a powerful line of antiviral defence called intrinsic immunity, which includes proteins referred to as restriction factors. In the case of HIV-1, several restriction factors have been identified that inhibit the viral life cycle in different ways: APOBEC3G induces G-to-A hypermutation of the viral genome; TRIM5 α perturbs uncoating of viral cores; BST2/tetherin retains viral particles at the surface of infected cells; IFITM family proteins inhibit membrane hemifusion and viral entry; and Schlafen11 perturbs HIV protein synthesis by binding to tRNAs and altering codon usage. Finally, the dNTP hydrolase SAMHD1 acts to deplete the intracellular pool of nucleotides available for viral DNA synthesis by reverse transcriptase (RT) (Hrecka *et al.*, 2011; Laguette *et al.*, 2011; Lahouassa *et al.*, 2012). HIV has evolved strategies to overcome the imposed replicative block by most of these restriction factors, often by triggering proteasomal degradation of the inhibitory factor. In the case of SAMHD1, the Vpx protein encoded by both HIV-2 and SIV hijacks a host cell ubiquitin ligase complex to target SAMHD1 for degradation. In contrast, HIV-1 lacks Vpx and is therefore unable to overcome SAMHD1 restriction, explaining why infection of myeloid cells and resting CD4⁺ T cells is a poorly efficient process (Baldauf *et al.*, 2012; Descours *et al.*, 2012). While it is unclear why HIV-1 did not evolve anti-SAMHD1 strategies too, reduced HIV-1 infection of dendritic cells appears to be beneficial by allowing viral escape from immune detection (Puigdomenech *et al.*, 2013). In addition to HIV-1, a large panel of other retroviruses (Gramberg *et al.*, 2013) as well as DNA viruses such as vaccinia and herpes viruses (Hollenbaugh *et al.*, 2013) are also restricted by SAMHD1 (Figure 1).

Before being identified as an antiviral factor, SAMHD1 was already known to be mutated in the Aicardi-Goutières syndrome (AGS), an inherited autoimmune encephalopathy that can also be caused by mutations in other nucleic acid-processing enzymes (TREX1, ADAR1 and RNASEH2). This inflammatory disorder induces symptoms similar to those observed during a congenital infection, and is associated with exacerbated secretion of type-I interferon (IFN), probably caused by sensing of endogenous nucleic

	HUMAN	MOUSE

Genes causing Aicardi Goutières syndrome-like symptoms or autoimmunity	<ul style="list-style-type: none"> • TREX1 • ADAR1 • SAMHD1 • RNASEH2A • RNASEH2B • RNASEH2C 	Yes • TREX1 • ADAR1 No • SAMHD1 • RNASEH2B • RNASEH2C
dNTP content in wild-type macrophages	Low 0.05 μ M	High 0.5 μ M
SAMHD1 effect on dNTPs	5- to 30-fold decrease in resting cells	5-fold decrease in resting cells
Viruses restricted by SAMHD1	Retroviruses • HIV • FIV • RSV • MPMV • EIAV DNA viruses • Vaccinia virus • Herpes simplex virus 1	Modified HIV vector
Other features of SAMHD1	<ul style="list-style-type: none"> • Nucleic acid binding and cleavage • Link with some cancers • Negatively regulated by phosphorylation 	ND

Figure 1 Comparison of AGS-causing genes, macrophage dNTP levels and SAMHD1 effects between humans and mice.

acids. More recently, SAMHD1 has also been linked to cancer, for instance in studies aimed at identifying genes involved in leukaemia (Schuh *et al*, 2012; De Silva *et al*, 2013).

So far, it has remained unknown whether SAMHD1 blocks retroviral infection and reduces the intracellular dNTP pool also *in vivo*. To address these questions, Rehwinkel *et al* (2013) now generated SAMHD1-deficient mice. These mice indeed display higher dNTP levels than WT mice. While plasma levels of type-I IFN were not affected by the absence of SAMHD1, the authors could observe an 'interferon signature' consisting of upregulation of IFIT2, TNF α and other interferon-stimulated genes (ISGs) in spleen and macrophages. Surprisingly, SAMHD1^{-/-} mice were healthy and did not develop AGS (Figure 1), implying that additional exogenous events (such as microbial infection) or mutations in additional genes may be required to trigger AGS-like symptoms in mice. Nevertheless, it is interesting to compare the phenotypes of the mouse knockout models that are now available for all genes known to cause AGS in humans (reviewed in Behrendt and Roers, 2013): TREX1^{-/-} mice develop autoimmune myocarditis and produce high levels of type-I IFN, a phenotype that depends on the IFNAR/IRF3/STING pathway and that is rescued by treatment with antiretroviral compounds; it has been proposed that TREX1^{-/-} mice accumulate retroelement-derived nucleic acids that trigger IFN α overproduction, ultimately leading to disease. ADAR1 deletion causes embryonic lethality in mice, but hematopoietic stem cells and other tissues derived from ADAR1^{-/-} embryos also displayed a clear induction of ISGs. Deletion of RNASEH2 subunits too is embryonic lethal and induces massive DNA damage; consistent with this critical genome integrity role, all AGS mutations in human RNASEH2 are hypomorphic and do not completely abrogate the enzyme's activity. Therefore, the available mouse models only partly recapitulate the clinical and biological features of humans suffering from AGS, with the typical IFN signature and inflammation observed in TREX1^{-/-} and ADAR1^{-/-} mice but not in SAMHD1^{-/-} and RNASEH2-mutated mice. Future work should help elucidating the basis for these different phenotypes, and determining what additional events may be required to trigger AGS in mice devoid of SAMHD1 or RNASEH2.

The other surprising result of the study by Rehwinkel *et al* (2013) is that infection with a regular HIV-1 vector was not affected by SAMHD1 in mice. Likewise, the levels of replication of various tested exogenous murine retroviruses (such as Moloney murine leukaemia virus) and endogenous murine retroelements were equivalent in WT and SAMHD1^{-/-} animals, suggesting that dNTPs may not be limiting for their replication in this mouse model. However, SAMHD1^{-/-} mice displayed increased susceptibility to infection when challenged with a modified HIV-1 vector that carries a mutant RT version with a lower affinity for dNTPs. The same was true for primary dendritic cells, macrophages and fibroblasts from SAMHD1-

deficient mice, indicating that this mutant HIV vector is in fact restricted by murine SAMHD1. These observations could be explained by quantifying cellular dTTP levels in normal and knockout animals: unexpectedly, wild-type murine macrophages contained 0.5 μ M dTTP, a concentration already ten-fold greater than that reported previously for human cells (Lahouassa *et al*, 2012). dTTP levels are even higher in SAMHD1^{-/-} mice, reaching 2.5 μ M. This helps understanding why mutant but not wild-type HIV-1 vector was restricted by SAMHD1, since the nucleotide affinity of wild-type HIV-1 RT ($K_M = 0.07 \mu$ M) would make it maximally active even in the presence of the basal dNTP level in wild-type murine macrophages, whereas the mutated RT used in this study displays optimal activity in the presence of 1.0–2.5 μ M dTTP (Diamond *et al*, 2003), and therefore benefits from increased levels in SAMHD1-deficient cells. Parallel quantifications of dNTP levels in cells from both species will be required to confirm that mice have a higher dNTP pool than humans and further validate these conclusions.

Therefore, while this study provides a proof-of-concept that SAMHD1 can exert antiviral effects *in vivo* by reducing the dNTP pools, the exogenous and endogenous murine retroviruses tested in this study were not affected by the absence of SAMHD1. It also remains unclear if SAMHD1 has a direct effect on reverse transcription *in vivo*, or whether it indirectly inhibits HIV transduction by inducing an antiviral state. To determine which step of the HIV cycle SAMHD1 affects in mice, it will also be worthwhile to quantify viral nucleic acid levels during infection. The new mouse model thus provides a unique tool to further characterize the role of SAMHD1 *in vivo*, for instance by monitoring viral replication and host responses after challenge with other retroviruses and DNA viruses.

Why normal mice seem to have such comparably high levels of dNTPs remains an intriguing question. It is probably not the result of reduced activity of murine SAMHD1: when expressed in human cell lines, its efficiency to decrease dNTP levels is similar to the human enzyme (Lahouassa *et al*, 2012). Irrespective of that, the large dNTP pool in mice could be the reason why SAMHD1^{-/-} mice do not develop autoimmune disease. It could further allow replication of endogenous retroelements and impact genome stability, explaining why some endogenous retroviruses are more active in mice than in humans. Furthermore, understanding why SAMHD1^{-/-} mice do not develop autoimmune disease will be another key question important for the understanding of the pathophysiology of AGS. This model, even if it only partially mimics the properties of human SAMHD1, will be an important tool to understand the function of SAMHD1 under normal and pathological situations such as autoimmune and inflammatory diseases, cancer and AIDS.

Conflict of interest

The authors declare that they have no conflict of interest.

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