THE EMBO JOURNAL

Manuscript EMBO-2013-85370

SAMHD1-dependent retroviral control and escape in mice

Jan Rehwinkel, Jonathan Maelfait, Anne Bridgeman, Rachel Rigby, Bruce Hayward, Rachel A. Liberatore, Paul D. Bieniasz, Greg J. Towers, Luis F. Moita, Yanick J. Crow, David T. Bonthron and Caetano Reis e Sousa

Corresponding author: Caetano Reis e Sousa, Cancer Research UK

Review timeline:

Submission date:	16 April 2013
Editorial Decision:	07 May 2013
Revision received:	26 June 2013
Accepted:	01 July 2013

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

Editor: Karin Dumstrei

1st Editorial Decision 07 May 2013

Thank you for submitting your manuscript to the EMBO Journal. Your study has now been seen by two referees and their comments are provided below.

As you can see both referees find the study interesting and support publication in The EMBO Journal. While referee #2 is happy with the paper as is, referee #1 raises a number of concerns. Given the positive comments I would like to invite a suitably revised paper. The concerns raised by referee #1 are very reasonable and straight forward, but I think it would be helpful to discuss upfront what is exactly needed to address in the revised version. We can do so by email.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for submitting your interesting study for publication in the EMBO Journal.

REFEREE REPORTS

Referee #1

Rehwinkel et al. generated transgenic mice deleted for the SAMHD1 gene, which has been previously linked to Aicardi-Goutieres Syndrome and restriction of HIV-1. SAMHD1-/- mice were healthy, but displayed enhanced levels of interferon in the spleen. The levels of dTTP were also increased in SAMHD1-depleted cells, confirming SAMHD1 deoxynucleotide triphosphohydrolase activity in vivo. However, the authors did not detect restriction of WT HIV-1 or of murine

retroviruses by SAMHD1 in vivo: only a HIV RT mutant with lower affinity for dNTPs was restricted. This result raises many questions, which were not fully addressed in this manuscript. Overall, experiments are well performed and the results clearly presented, additional experiments will increase the relevance of this interesting work.

Major concerns :

1. Fig3. : An absolute quantification of dNTPs levels is missing. Showing the concentration in pg.mL-1 would allow to directly compare the results to those of Lahouassa et al. 2012, in which the levels of dNTPs are measured in SAMHD1 positive or negative human cells. Moreover, the authors only showed the levels of dTTP. Did they quantify other nucleotides ? This is important because Lahouassa et al. observed that SAMHD1 impact differently the levels of each dNTP - dTTP being the most sensitive.

2. In vitro experiments in cell cultures should help defining whether murine and human SAMHD1 are similarly effective in reducing the dNTP pool.

3. In Fig.4 and Fig5., the authors show that SAMHD1 has no impact on infection by several murine retroviruses or on retroelements. Is the Km of the RT enzyme of these viruses known ? are levels of type-I interferon increased in samhd1-null mice during retroviral infection or upon transduction with VSV-pseudotyped lentivirus? Fig. S3B, it is shown that CD11c+ cells are similarly transduced with or without SAMHD1. What are the levels of type-I interferon released in this system?

4. Figure 7c : the levels of infection in macrophages are unusually high (37.5% in SAMHD1+/+ mice) in contrast of the other cell types (roughly 1%). In presence of SAMHD1, macrophages are not expected to be much more sensitive to HIV-1 infection than DCs. How do the authors explain this result?

5. Fig.7c : HIV-1 WT infection is decreased in macrophages of SAMHD1 null mice (25.6% vs 37.5%). In Fig.7D, this difference is no longer visible.

6. The authors should include the experiments of BM-DM infection displayed in Fig.S3A in the main manuscript, together with Fig.6, and use the V1481 mutant with BM-DM as well.

7. In Fig.7, the authors show a 10-fold increase of V148I replication in T cells. Did they monitor the activation state of the T cells?

8. The explanation provided for the lack of restriction of WT HIV-1 is not fully convincing : if co-evolution with SAMHD1 selected for HIV-1 with decreased sensitivity to restriction, then the same absence of phenotype would have been observed in human cells. Many groups observed efficient restriction of WT HIV-1 by SAMHD1, with many viral strains. To better explain this surprising result, the authors could address some of the following questions :

- Are the dNTP levels high in SAMHD1+/+ murine cells, compared to those in human cells ?

- Is there IFN production following HIV-1 infection in this model ?

- How is the sequence of murine SAMHD1 compared to the human counterpart?

- Does murine SAMHD1 actually bind and degrade nucleic acids as suggested by Goncalves et al. 2012, Beloglazova et al. 2013 ?

- What could be the role of phosphorylation of T592 on murine SAMHD1, as recently described for the human molecule (Cribier et al. 2013, White et al. 2013) ?

Minor point.
Litterature to quote:
- Page 1. It is stated that human DCs are permissive to HIV-2. This remains controversial (see Duvall JV 2007).
- SAMHD1 decreases type-I interferon production in human DCs and macrophages (Berger,

PlosPath 2011, Puigdomenech, JV 2013)

Referee #2

SAMHD1 is a triphosphohydrolase that acts as a host restriction factor that blocks reverse transcription of retroviral genome by limiting the availability of the free dNTPs that are needed for cDNA synthesis. SAMDH1 action is inhibited by the Vpx viral protein. In contrast to HIV-2, HIV-1 does not encode Vpx and thus human DCs are not productively infected. The present study addresses the role of SAMHD1 in vivo and analyzes whether Samhd1 null mice accumulate retroviral nucleic acid products that chronically trigger IFN production. Samhd1 null mice do not express detectable amounts of circulating IFN. Although they display spontaneous type I interferon signatures in spleen, macrophages and fibroblasts Samhd1 null mice were healthy. Moreover, Samhd1-/- cells mounted normal IFN responses upon triggering by nucleic acid or virus infection. As expected, SAMHD1-deficient bone marrow-derived DCs and MFs have elevated dNTP levels but this does not translate into 1/ increased viral load following Mo-MLV infection 2/ increased RNA levels of endogenous retroelements and 3/ increased infection with VSV-G-pseudotyped HIV-1 vectors. However, by « sensitizing » the system with an HIV-1 vector mutant bearing a reverse transcriptase with a lower affinity for dNTP binding, SAMDH1-deficient cultured cells and lymphoid and non-lymphoid populations from SAMDH1-deficient mice became 3 to 5 fold more sensitive to the mutant virus. Therefore, this straightforward study demonstrates that SAMHD1 can restrict lentiviruses in vivo.

Minor point
Reference Young et al. is in part missing.

1st Revision - authors' response 26 June 2013

Referee #1

Rehwinkel et al. generated transgenic mice deleted for the SAMHD1 gene, which has been previously linked to Aicardi-Goutieres Syndrome and restriction of HIV-1. SAMHD1-/- mice were healthy, but displayed enhanced levels of interferon in the spleen. The levels of dTTP were also increased in SAMHD1-depleted cells, confirming SAMHD1 deoxynucleotide triphosphohydrolase activity in vivo. However, the authors did not detect restriction of WT HIV-1 or of murine retroviruses by SAMHD1 in vivo: only a HIV RT mutant with lower affinity for dNTPs was restricted. This result raises many questions, which were not fully addressed in this manuscript. Overall, experiments are well performed and the results clearly presented, additional experiments will increase the relevance of this interesting work.

We thank the reviewer for his/her positive comments on our work. We believe that additional experiments now included in the revised version of our manuscript strengthen its relevance as detailed below.

Major concerns :

1. Fig3. : An absolute quantification of dNTPs levels is missing. Showing the concentration in pg.mL-1 would allow to directly compare the results to those of Lahouassa et al. 2012, in which the levels of dNTPs are measured in SAMHD1 positive or negative human cells. Moreover, the authors only showed the levels of dTTP. Did they quantify other nucleotides ? This is important because Lahouassa et al. observed that SAMHD1 impact differently the levels of each dNTP - dTTP being the most sensitive.

We have performed additional experiments that are presented in a new Fig. 3. We now show that the levels of all four dNTPs are elevated in *Samhd1*$^{-/-}$ cells. Moreover, we have quantified dTTP levels. These results are presented in Fig. 3F and are shown in mM. This allows direct comparison with the data for human monocyte derived macrophages published by Lahouassa et al. (Nature Immunology 2012, Fig. 2c). Remarkably, dTTP concentrations in wild-type mouse BM-DCs are around one order of magnitude greater than those found in human macrophages. They also exceed the K_M of wild-

type HIV-1 RT. Therefore, dNTP supply is not limiting for reverse transcription in wild-type mouse cells. This explains our observation that only a point-mutant virus with decreased binding of RT to dNTPs is restricted by SAMHD1 in mouse cells. We believe that these results provide a mechanistic explanation for our findings and significantly strengthen our conclusions, as now stated in the revised discussion. We thank the reviewer for encouraging us to pursue these experiments.

2. In vitro experiments in cell cultures should help defining whether murine and human SAMHD1 are similarly effective in reducing the dNTP pool.

When mouse SAMHD1 was overexpressed in human U937 cells, it was equally efficient at reducing dNTP levels compared to the human protein (Lahouassa et al., Nature Immunol 2012, Fig 3b). We now refer to this point in the discussion. Rather than being attributable to differences in SAMHD1 efficiency, we think that our data can be explained by the size of the dNTP pool in mouse vs. human cells, as discussed above. Why mouse BM-DCs contain more dNTPs than human MDMs is an interesting question and may depend on many factors, including basic parameters of metabolic control, cell type and differentiation status.

3. In Fig.4 and Fig5., the authors show that SAMHD1 has no impact on infection by several murine retroviruses or on retroelements. Is the Km of the RT enzyme of these viruses known?

Compared to the HIV-1 polymerase, the MoMLV enzyme has a lower affinity for dNTPs: HIV-1 RT has K_M values for dNTPs ranging from 0.0328 to 0.128 mM, whereas the MLV enzyme has K_M values ranging from 4.96 to 29.2 mM (Diamond et al., JBC 2004). However, this virus replicates in cycling lymphocytes that have very high dNTP levels, providing a likely explanation for the lack of SAMHD1-restriction. The K_M values of the RTs of retroelements are unknown. We now refer to these points in the revised discussion.

are levels of type-I interferon increased in samhd1-null mice during retroviral infection or upon transduction with VSV-pseudotyped lentivirus? Fig. S3B, it is shown that CD11c+ cells are similarly transduced with or without SAMHD1. What are the levels of type-I interferon released in this system?

Replication-deficient lentiviral vectors, similar to the ones used in our study, did not induce type I interferon in infected human cells depleted of SAMHD1 by Vpx-delivery (Manel et al., Nature 2010, Fig. S7B). Similarly, we have not been able to detect interferon production in *Samhd1*$^{+/+}$ and *Samhd1*$^{-/-}$ mouse cells following infection. These data are now included in the manuscript (Fig. S3) and we have inserted a comment to this effect in the discussion.

4. Figure 7c : the levels of infection in macrophages are unusually high (37.5% in SAMHD1+/+ mice) in contrast of the other cell types (roughly 1%). In presence of SAMHD1, macrophages are not expected to be much more sensitive to HIV-1 infection than DCs. How do the authors explain this result?

Similarly high rates of macrophage transduction have been reported (for example, van den Brand et al., PLOS ONE 2013). A possible explanation is that these cells are highly phagocytic and might therefore take up more virus than other cells.

5. Fig.7c : HIV-1 WT infection is decreased in macrophages of SAMHD1 null mice (25.6% vs 37.5%). In Fig.7D, this difference is no longer visible.

Samples from multiple mice were analyzed. Fig 7c shows an example. Fig 7d displays the average and standard deviation for all samples and shows that there are no statistically significant differences.

6. The authors should include the experiments of BM-DM infection displayed in Fig.S3A in the main manuscript, together with Fig.6, and use the V1481 mutant with BM-DM as well.

As requested, we have moved Fig. S3A into the main manuscript. We have used the V148I mutant virus in BM-DCs, fibroblasts and *in vivo*. We therefore feel that repeating the experiments in Fig. 6

with BM-DMs would not add much to the manuscript. After consultation with the editor, we decided not to embark on these experiments.

7. In Fig.7, the authors show a 10-fold increase of V148I replication in T cells. Did they monitor the activation state of the T cells?

We have not monitored the activation state of the T cells. We believe that studies on the impact of SAMHD1-deficiency on adaptive immune responses to lentivirus infection are beyond the scope of this manuscript.

8. The explanation provided for the lack of restriction of WT HIV-1 is not fully convincing : if co-evolution with SAMHD1 selected for HIV-1 with decreased sensitivity to restriction, then the same absence of phenotype would have been observed in human cells. Many groups observed efficient restriction of WT HIV-1 by SAMHD1, with many viral strains.

We agree with the reviewer: many groups have observed restriction of WT HIV-1 by SAMHD1 in human cells. However, it is noteworthy that this is not an absolute restriction. Indeed, a fraction of cells is infected in the presence of SAMHD1. For example, Laguette et al. (Nature 2011, Fig 3d) found that 4% of human monocyte derived DCs were infected in SAMHD1-sufficient control cells. This increased around 6-fold when SAMHD1 was depleted. Moreover, the study from Duvall et al. (JV 2007) - pointed out by the reviewer below - showed that human DCs can be infected by HIV-1. Similarly, many papers in the field showed that human myeloid cells can be infected by HIV-1 in the presence of SAMHD1, albeit often at very low infection rates.
In addition to the data presented in our manuscript, the following lines of evidence support our interpretation that HIV-1 counteracts SAMHD1-restriction by means of a polymerase with high dNTP affinity:

- The V148I RT mutant virus used here was around 6-fold less infective in SAMHD1-sufficient human monocyte derived macrophages than the WT virus (Lahouassa et al., Nature Immunology 2012, Figure 4a). This observation is in line with our data and also shows that SAMHD1 restriction of WT HIV-1 in human cells is partial and is governed by dNTP availability.
- In a primer extension assay, HIV-1 RT was found to be more efficient at low dNTP concentrations than HIV-2's polymerase (Boyer, JV 2012). At the same time, only HIV-2 encodes the SAMHD1 antagonist Vpx.

Taken together, both SAMHD1-dependent restriction and RT-dependent escape are partial. To highlight this, the discussion now reads: "Our data suggest that HIV-1's strategy for **partially** overcoming restriction by SAMHD1…". We have also inserted: "As such, HIV-1 partially escapes SAMHD1 restriction and in many instances is able infect at least a small proportion of human myeloid cells (Duvall et al, 2007; Hrecka et al, 2011; Laguette et al, 2011; Lahouassa et al, 2012)."

To better explain this surprising result, the authors could address some of the following questions :

- Are the dNTP levels high in SAMHD1+/+ murine cells, compared to those in human cells ?

As the reviewer correctly surmised high dNTP levels in mouse vs. human cells provides a mechanistic explanation for the lack of restriction of WT HIV-1 (see point 1&2). We thank the reviewer for encouraging us to address this point.

- Is there IFN production following HIV-1 infection in this model ?

see point 3

- How is the sequence of murine SAMHD1 compared to the human counterpart?

We have included an alignment of human and mouse SAMHD1 proteins in the revised manuscript (Fig. S8). The two proteins share around 70% amino acid identity and a statement about similarities and differences between them has now been included in the discussion.

- Does murine SAMHD1 actually bind and degrade nucleic acids as suggested by Goncalves et al. 2012, Beloglazova et al. 2013 ?

- What could be the role of phosphorylation of T592 on murine SAMHD1, as recently described for the human molecule (Cribier et al. 2013, White et al. 2013) ?

Whether mouse SAMHD1 has nuclease activity and is regulated by phosphorylation are important questions, but we feel that these are beyond the scope of our study. For completeness, we have included and briefly discussed these references in the revised manuscript.

Minor point.
Litterature to quote:
- Page 1. It is stated that human DCs are permissive to HIV-2. This remains controversial (see Duvall JV 2007).

We thank the reviewer for pointing this out and have removed this statement from the introduction. This reference has been included in the discussion.

- SAMHD1 decreases type-I interferon production in human DCs and macrophages (Berger, PlosPath 2011, Puigdomenech, JV 2013)

These references have now been included in the discussion.

Referee #2

SAMHD1 is a triphosphohydrolase that acts as a host restriction factor that blocks reverse transcription of retroviral genome by limiting the availability of the free dNTPs that are needed for cDNA synthesis. SAMDH1 action is inhibited by the Vpx viral protein. In contrast to HIV-2, HIV-1 does not encode Vpx and thus human DCs are not productively infected. The present study addresses the role of SAMHD1 in vivo and analyzes whether Samhd1 null mice accumulate retroviral nucleic acid products that chronically trigger IFN production. Samhd1 null mice do not express detectable amounts of circulating IFN. Although they display spontaneous type I interferon signatures in spleen, macrophages and fibroblasts Samhd1 null mice were healthy. Moreover, Samhd1-/- cells mounted normal IFN responses upon triggering by nucleic acid or virus infection. As expected, SAMHD1-deficient bone marrow-derived DCs and MFs have elevated dNTP levels but this does not translate into 1/ increased viral load following Mo-MLV infection 2/ increased RNA levels of endogenous retroelements and 3/ increased infection with VSV-G-pseudotyped HIV-1 vectors. However, by « sensitizing » the system with an HIV-1 vector mutant bearing a reverse transcriptase with a lower affinity for dNTP binding, SAMDH1-deficient cultured cells and lymphoid and non-lymphoid populations from SAMDH1-deficient mice became 3 to 5 fold more sensitive to the mutant virus. Therefore, this straightforward study demonstrates that SAMHD1 can restrict lentiviruses in vivo.

We thank the reviewer for his/her positive evaluation of our manuscript.

Minor point
Reference Young et al. is in part missing.

We thank the reviewer for pointing this out. The reference is now complete.

Other changes

To reflect the authors' contributions to the revised version of the manuscript, Jonathan Maelfait is now second author and Anne Bridgeman third author.

Accepted 01 July 2013

Thank you for submitting your revised manuscript to The EMBO Journal. Your revised version has now been seen by referee #1 and as you can see below the referee is happy with the introduced changes and support publication here. I am therefore very pleased to accept the paper for publication here.

Please see below for important information on how to proceed. Make sure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

We also need one merged file of the supplemental data. You can send it via email.

Thank you for contributing to the EMBO Journal!

REFEREE REPORT

Referee #1

The authors have convincingly addressed my concerns. This is a very nice work.

