

Reply to Pauls et al.: p21 is a master regulator of HIV replication in macrophages through dNTP synthesis block

Awatef Allouch, Annie David, Sarah M. Amie, Hichem Lahouassa, Loic Chartier, Florence Margottin-Goguet, Françoise Barré-Sinoussi, Baek Kim, Asier Saez-Cirion, Gianfranco Pancino

► To cite this version:

Awatef Allouch, Annie David, Sarah M. Amie, Hichem Lahouassa, Loic Chartier, et al.. Reply to Pauls et al.: p21 is a master regulator of HIV replication in macrophages through dNTP synthesis block. Proceedings of the National Academy of Sciences of the United States of America , National Academy of Sciences, 2014, 111 (14), pp.E1325–1326. 10.1073/pnas.1322699111 . pasteur-01420528

HAL Id: pasteur-01420528

<https://hal-pasteur.archives-ouvertes.fr/pasteur-01420528>

Submitted on 16 May 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Response to Pauls E. et al. :

p21: a master regulator of HIV replication in macrophages through dNTP synthesis block

We thank Pauls and colleagues for their comment (1) on our article describing that p21 restricts reverse transcription of HIV-1 in monocyte derived macrophages (MDM) by blocking the synthesis of dNTPs through the repression of the transcription factor E2F1 and the subsequent inhibition of the expression of RNR2 (2). Pauls et al confirm our finding that p21 blocks HIV replication in MDM, further show that p21 regulates the phosphorylation of SAMHD1 in MDM and suggest that p21-mediated restriction in these cells is dependent on SAMHD1. Indeed it has been recently reported that SAMHD1 viral restriction activity, but not its dNTPase activity, is inhibited by CDK1-mediated phosphorylation at residue T592 (3-5). Like Pauls et al, we have found that p21 does regulate SAMHD1 phosphorylation in MDM (Fig. 1). However, we believe that p21 restriction is, at least in MDM, mainly mediated by the block of dNTP synthesis due to RNR2 suppression, which is upstream and unrelated to the dNTP catabolic activity of SAMHD1. The crucial role of RNR2 is also supported by the almost complete (94%-98%) inhibition of HIV-1 and SIVmac reverse transcription by siRNA-mediated knockdown of RNR2 (2). In addition, p21-mediated HIV-restriction in MDM is overcome by addition of dNs to the culture medium (2), which is in agreement with a restriction caused by a deficit of intracellular dNTPs.

Pauls et al. show that, after MDM treatment with viral like particles carrying Vpx (VLP_{Vpx}), which degrade SAMHD1, siRNA-mediated knockdown of p21 failed to enhance MDM transduction of a VSV-pseudotyped HIV-1 (Fig. 2H of the Letter). However, in this experiment, the increase of HIV-1 transduction upon p21 silencing in untreated MDM is very weak, less than 2 fold, and this effect might be masked by the strong increase of HIV-1 transduction caused by VLP_{Vpx}.

We have shown that p21-mediated restriction in MDM affects Vpx-bearing lentiviruses such as HIV-2 and SIVmac, and therefore it is still effective after degradation of the restriction factor

SAMHD1 by physiological amounts of Vpx (2, 6). In addition, we show here that HIV-1 transduction is actually increased by SIVmac251 co-infection (300 ng p27/10⁶ MDM) and subsequent degradation of SAMHD1, but p21 induction by IVIg treatment still causes strong HIV-1 inhibition (Fig. 2).

It should be noted that the experimental system used by Pauls et al presents some relevant differences with ours: while we differentiated MDM for 7 days in presence of human serum, Pauls et al. differentiated monocytes with MCSF just for 3 days, which appears insufficient to obtain differentiated macrophages. They claim that MDM at that time were proliferating and that p21 silencing increased the number of proliferating cells (fig. 1A, B of the Letter). The differences reported in proliferating cells (15.1% vs 12.1% and 23.7% vs 20.4%, for cells with our without p21 knockdown respectively in 2 donors) seem hardly significant, and no link is provided between increased viral replication upon p21 knockdown and the increased frequency observed in proliferating cells.

In summary, our observations suggest that p21 restricts HIV-1 reverse transcription through the block of dNTP biosynthesis pathway in MDM, upstream to SAMHD1-mediated dNTP depletion. This does not exclude further antiviral activity of p21 through inhibition of SAMHD1 phosphorylation, as we had already suggested in the discussion of our article (2).

References

1. Este et al (2014) p21 regulates the HIV-1 restriction factor SAMHD1. Submitted to Proc Natl Acad Sci USA
2. Allouch A, *et al.* (2013) p21-mediated RNR2 repression restricts HIV-1 replication in macrophages by inhibiting dNTP biosynthesis pathway. *Proceedings of the National Academy of Sciences of the United States of America* 110(42):E3997-4006.
3. White TE, *et al.* (2013) The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation. *Cell host &*

microbe 13(4):441-451.

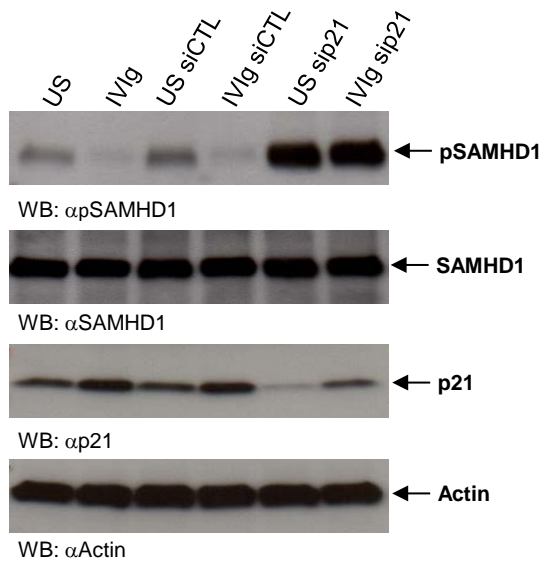
4. Cribier A, Descours B, Valadao AL, Laguette N, & Benkirane M (2013) Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. *Cell reports* 3(4):1036-1043.
5. Welbourn S, Dutta SM, Semmes OJ, & Strebel K (2013) Restriction of virus infection but not catalytic dNTPase activity is regulated by phosphorylation of SAMHD1. *Journal of virology* 87(21):11516-11524.
6. Bergamaschi A, *et al.* (2009) The CDK inhibitor p21Cip1/WAF1 is induced by FcγR activation and restricts the replication of human immunodeficiency virus type 1 and related primate lentiviruses in human macrophages. *Journal of virology* 83(23):12253-12265.

Figure Legends

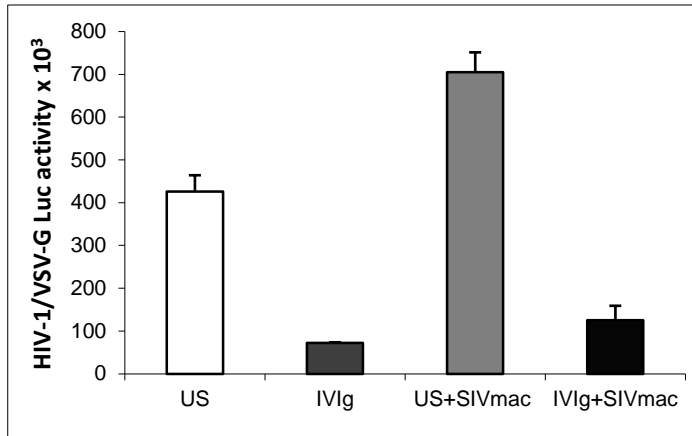
Figure 1. p21 modulates negatively the phosphorylation of SAMHD1 in macrophages. Untimulated (US) or IVIg-activated MDM were silenced for p21 with smart pool small interfering RNA (siRNA) (Dharmacon) against p21 gene (sip21). Control cells were transfected with a non-targeting pool siRNAs (siCTL). At 24 hours post IVIg stimulation and 22 hours post-silencing, macrophages were analyzed by western blot (WB) for the expressions of phosphorylated SAMHD1 (pSAMHD1), SAMHD1, p21 and Actin using the indicated antibodies. Data are representative of results obtained from MDM from three different blood donors. The anti-pSAMHD1 antibody was a kind gift of Moncef Benkirane, Institut de Génétique Humaine, CNRS UPR1142, Montpellier, France.

Figure 2. The co-infection of macrophages with SIVmac enhances HIV-1 replication but does not relieve the p21 restriction barrier. Unstimulated (US) or immobilized immunoglobulin-activated (IVIg) monocyte-derived macrophages (MDM) from two different blood donors were infected at 24 hours after IVIg stimulation with HIV-1.luc/VSVG, expressing *luciferase* as reporter gene, and co-infected or not with SIV-mac (300 ng p27 for 1×10^6 MDM). HIV-1 replication was quantified by

measuring luciferase (Luc) activity at 72 hours post-infections. The data are means \pm SD of triplicate wells.



Donor 1



Donor 2

