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

We thank Pauls et al. for their comments (1) on our article describing that p21 restricts reverse transcription of HIV-1 in monocyte-derived macrophages (MDM) by blocking the synthesis of deoxynucleotides (dNTPs) through the repression of the transcription factor E2F1 and the subsequent inhibition of the expression of ribonucleotide reductase subunit R2 (RNR2) (2). Pauls et al. (1) confirm our finding that p21 blocks HIV replication in MDM, further show that p21 regulates the phosphorylation of SAM domain and HD domain-containing protein 1 (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. (1), 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 because of 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 simian immunodeficiency virus (SIV)mac 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. (1) show that, after MDM treatment with viral-like particles (VLP) carrying lentiviral virus protein x (Vpx) (VLP$_{Vpx}$), which degrade SAMHD1, siRNA-mediated knockdown of p21 failed to enhance MDM transduction of a VSV-pseudotyped HIV-1 (figure 2h in ref. 1). However, in this experiment, the increase of HIV-1 transduction upon p21 silencing in untreated MDM is very weak, less than twofold, 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 coinfection (300 ng p27/10^6 MDM) and subsequent degradation of SAMHD1, but p21 induction by immobilized Ig (IVIg) treatment still causes strong HIV-1 inhibition (Fig. 2).

It should be noted that the experimental system used by Pauls et al. (1) presents some relevant differences with ours: Although we differentiated MDM for 7 d in the presence of human serum, Pauls et al. differentiated monocytes with M-CSF for just 3 d, which appears insufficient to obtain differentiated macrophages. Pauls et al. claim that MDM at that time were proliferating and that p21 silencing increased the number of proliferating cells (figure 1 a and b in ref. 1). 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 two 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 finding does not exclude further antiviral activity of p21 through inhibition of SAMHD1 phosphorylation, as we had already suggested in the Discussion section of our article (2).

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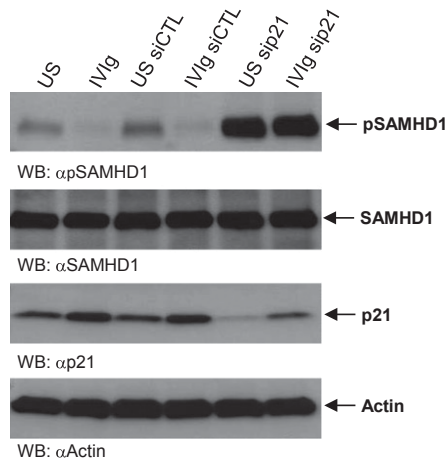
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Fig. 1. p21 modulates negatively the phosphorylation of SAMHD1 in macrophages. Untimulated (US) or immobilized immunoglobulin (IVIg)-activated MDM were silenced for p21 with smart pool containing four small interfering RNA (siRNA) (Dharmacon) against the p21 gene (sip21). Control cells were transfected with a nontargetting pool siRNAs (siCTL). At 24 h post-IVIg stimulation and 22 h postsilencing, 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 Monsef Benkirane (Institut de Génétique Humaine, Centre National de la Recherche Scientifique Unité Propre de Recherche 1142, Montpellier, France).
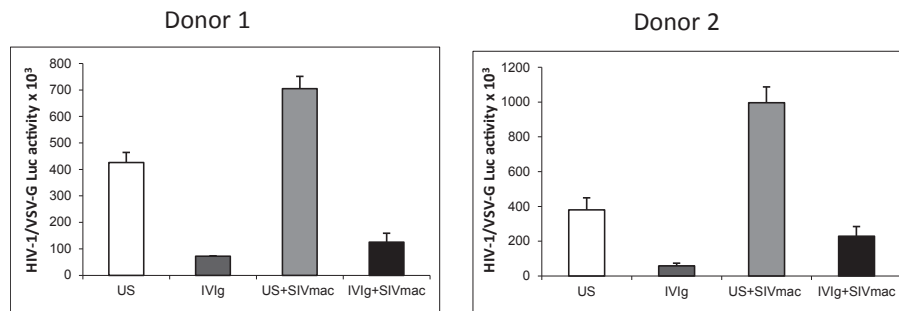

Fig. 2. The coinfection of macrophages with SIV_{mac}(Vpx+) enhances HIV-1 replication but does not relieve the p21 restriction barrier. Unstimulated (US) or IVIg-activated MDM from two different blood donors were infected at 24 h after IVIg stimulation with HIV-1.luc/VSVG, expressing *luciferase* as reporter gene, and coinfected or not with SIVmac (300 ng p27 for 1×10^6 MDM). HIV-1 replication was quantified by measuring luciferase (Luc) activity at 72 h postinfections. The data are means $\pm$ SD of triplicate wells.

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